Genetics and Memory: An Overview and an Empirical Example Investigated with Functional and Structural Magnetic Resonance Imaging

Thesis

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Abbreviations

5-HT: 5-Hydroxy-Triptamine (Serotonin)
5-HTT: Serotonin-Transporter
AMPA: α-Amino-3-hydroxy-5-Methyl-4-isoxazole-Propion Acid
APOE: Apolipoprotein E
BA: Brodmann's Area
BDNF: Brain-Derived Neurotrophic Factor
CJD: Creutzfeldt-Jakob Disease
CREB: cyclic adenosine monophosphate responsive element binding protein
Cyp46: Cytochrome Pigment 450 46A1
DNA: Desoxy-Ribonuclein Acid
EEG: ElectroEncephaloGram
HRF: Hemodynamic Response Function
LTP: Long Term Potentiation
NAA: N-acetyl-aspartate
NCAM: Neuronal Cell Adhesion Molecule
NMDA: N-Methyl-D-Aspartate Receptor
PFC: Prefrontal Cortex
PRND: Prion Doppel Gene
PRNP: Prion Protein Gene
PrP^{C}: Cellular Prion Protein
PrP^{SC}: Scrapie (Pathological) Prion Protein
RNA: Ribonuclein Acid
mRNA: messenger RNA
tRNA: transfer RNA
SNP: Single Nucleotide Polymorphism
TSE: Transmissible Spongiform Encephalopathies (Prion Diseases)
VOI: Volume Of Interest

Contents

Zusammenfassung

Die vorliegende Arbeit gibt zuerst eine Einführung in die Genetik, beleuchtet zelluläre Mechanismen und Systemaspekte von Gedächtnis, und gibt einen Überblick über einige Gene, die mit Gedächtnis in Zusammenhang gebracht werden.

Im Zentrum steht ein empirischer Artikel über den Einfluss eines Polymorphismus (Met129Val) des Prion-Protein-Gens aufs Gedächtnis. Wir wollten Befunde unserer Kollaborationspartner replizieren und ergänzen, die sagen, dass homozygote Val/Val-Träger beim Gedächtnis von Wörtern über 24 Stunden, aber noch nicht nach fünf Minuten, einen Nachteil haben. Wir führten eine Studie mit funktioneller Magnetresonanztomographie (fMRI) durch mit 3 Gruppen von jungen, gesunden Versuchspersonen mit verschiedenen Prion-Genotypen (Met/Met, Val/Met und Val/Val). Diese waren so gut als möglich angeglichen in Geschlecht, Alter, Bildung, weiteren genetischen Polymorphismen und in der Gedächtnisleistung. Wir behandelten Gedächtnisleistung als Kontrollvariable und die fMRI-Aktivierung als abhängige Variable. Dies war wichtig für die Interpretation der Resultate, weil mehr Aktivierung ein Zeichen von erfolgreicherer Erinnerung oder von mehr Mühe beim Erinnern sein kann, und wir wollten beide Effekte trennen können, weswegen wir den ersten Faktor konstant hielten. Die Resultate waren wie erwartet: Val-Träger zeigten mehr Aktivierung beim Wiedererkennen der Wörter, und zwar transitiv (die Val/Val-Träger mehr als die Val/Met-Träger und diese mehr als die Met/Met-Träger). Ausserdem korrelierten die Aktivierungen innerhalb der Met/Met-Träger und Val/Met-Träger meistens positiv mit der Erinnerungsleistung, bei den Val/Val-Trägern hingegen vorwiegend negativ. Beide Beobachtungen galten gleichermassen 30 Minuten nach dem Lernen der Wörter wie 24 Stunden nach dem Lernen. Wir interpretierten sie dahingehend, dass Val-Träger schlechtere Gedächnis-Fähigkeiten haben, sobald der Abruf mehr als 5 Minuten später erfolgt als die Lernphase. Zusätzlich fanden wir eine höhere Konzentration von grauer Materie bei Val-Trägern als bei Met-Trägern, trotz statistisch nicht unterschiedlichen Hirngrössen. Wir wissen nicht woher dieser Unterschied kommt, aber er scheint die Befunde im funktionellen MRI nicht zu erklären, da sie qualitativ gleichbleiben, wenn man die Dichte der Grauen Materie als Kontrollvariable berücksichtigt.

Am Ende der Arbeit werden methodische Fragen der Genetikforschung und der Forschung über Gedächtnis mit fMRI erörtert, und es wurde versucht, den Forschungszweig kritisch zu bewerten.

Summary

This work first gives an introduction on genetics, on cellular and system aspects of memory and on genes that have been proposed to influence memory functions.

The main part is an empirical paper on the influence of a polymorphism (Met129Val) of the prion protein gene on memory. To replicate and complement an earlier finding of our collaboration partners, which states that homozygous Val/Val carriers have an impaired episodic memory performance for words over a time lag of 24 hours, but not yet five minutes after learning, we conducted a functional magnetic resonance imaging (fMRI) study with three genetic groups of twelve young, healthy (Met/Met, Val/Met and Val/Val) subjects each, which were as similar as possible with respect to sex, age, education, several other genetic polymorphisms and memory performance. We treated task performance as a control variable and the activation during the task as the dependent variable. This was important for the interpretation of the results, because more activation can be a sign of more retrieval success, but also of more labor to reach a certain performance, and we wanted to be able to match out the first factor and evaluate only the second. Our study mainly conformed to our hypotheses: Val carriers showed more activity to reach the same recognition performance, in a transitive way: Val/Val-carriers more than Val/Met carriers and these in turn more than Met/Met carriers. In addition, Met/Met and Val/Met carriers showed more positive than negative within group correlations between memory-related fMRI activity and successful performance, while Val/Val carriers showed more negative correlations than positive correlations between activation and successful performance. Both effects held true at word recognition both 30 minutes after learning and 24 hours after learning. We interpreted these findings in term of lesser memory abilities in Val-carriers than in Met-carriers for long term memory over more than five minutes. An addition was a higher grey-matter concentration in large parts of the brain in Val-carriers than in Met-carriers, despite statistically equal whole brain volumes. We do not know the significance of this effect, but we think it is unlikely that the functional MRI results are an artifact of this structural difference, because we calculated statistical group comparisons with grey matter density as a control variable, and this did not change the result pattern.

In the end of the work, methodological issues of genetic research in general and with fMRI of memory in particular are discussed, and the field as a whole is evaluated.

1. Introduction: Individual Differences and Genetics

Performance in higher cognitive functions varies greatly between individuals. One person speaks six different languages fluently, while another needs a very big effort to learn just one new language. Observers use two kinds of explanation for this phenomenon: Maybe the parents and grandparents of the first person were already very good or poor in learning languages (‘nature’ or genetic point of view) or he/she had excellent opportunities to learn languages since his parents moved every second year to another country. Of course most explanations are already a mixture between the two points of view: Maybe he/she was very motivated to learn languages, but part of this motivation stemmed from the fact that it was easy for him to deal with languages. The topic is hotly debated (primarily in the context of intelligence) and has political implications: If an ability can be improved by interaction with certain environments (training), it would be fair for all citizens to have the same possibilities of learning, while if an ability is 100% hereditary, it is probably more economic to give persons with higher abilities employments where these abilities are needed and to find different jobs for people with different abilities. Personality psychologists started to look for more exact operationalizations of the involved concepts and to gather objective data. First they postulated that psychological features (and as such, abilities) have to fulfill two criteria to be considered as traits: They must be stable (for example, joyfulness is no trait because it can be totally different tomorrow than today) and consistent over situations (for example, fear is not a trait, because you may fear spiders but not crowds). Based on sharpened methodology, personality psychologists try to calculate how important genetic influences contrasted to environmental influences are for a certain trait. For example, they use monozygotic twins and dizygotic twins to estimate *heritability* (percentage estimate of genetic influence) measures. If homozygotic twins correlate significantly more than dizygotic twins in a trait, then there is a genetic influence on this trait. If quantifying this with heritability measures, one needs assumptions to do so, for example ‘variances caused by

genetic and environmental factors are independent' (which is highly improbable) or 'different genes do not interact with each other' (also highly improbable). The calculated heritability values have another immanent problem: They depend on the variabilities of genes and environments in a population. In a population where everybody went through very good schools, only people with severe disturbances are not able to read, and a large percentage of analphabets will have a high load of genetic risk factors for dyslexia, so heritability of the trait "dyslectic" is rather high; in a different population where some people go to good schools and learn to read with adequate methods, while do not have this possibility, heritability of the same trait will be much lower. We can also learn from personality psychologists, that there are two different kinds of environmental factors during the development of a child (Asendorpf, 1996): Factors shared by different children of a family ('*shared* factors'), and those that differ between children of the same family ('*nonshared* factors'). An example for a nonshared factor would be if a mother speaks more with the oldest child than with the following children. This kind of research starts to become interesting if you look for nonsummative interaction between persons and environments: For example, environments can be 'hereditary' in the sense that persons are choosing a similar environment than his/her parents would have chosen, without having directly been taught to do so. Part of the relative stability of personality during adulthood can be explained by the fact that adults are rather able to choose their environment for themselves, so they show reactions to more similar situations, which looks like personality stability. We should keep this in mind if someone says that heritability of memory functions is about 52% (McClearn et al., 1997). For us, this number means that it makes sense to search for genes that influence memory functions, because even if that heritability value would be somewhat lower or higher, it is substantial enough to wonder which genes confer this genetic influence. Trying to do this, it starts to be difficult, but also interesting: The interactions of genome and environment can be situated in various organizational levels of the organism, and we have to understand something of these different levels of explanation before we are able to understand what it really means that "a gene is involved in memory". It is a cumbersome, but instructive path for a psychologist to deal with this biological concepts: Behaviorism and in a certain sense also cognitive psychology taught us that the mind is a "black box", and in the rest of this work we will try to open it. The first step is that we learn some basic things about genetics.

1.1 Genetics: Some Basics

1.1.1 The Genetic Code

Hereditary information is stored in *DNA* (desoxyribo-nuclein-acid) molecules (Knippers, 1997). There are 4 different *nucleotid* bases ('letters'), namely adenine (A), thymine (T), cytosine (C) and guanine (G). DNA is organized in a double-helix, where each strand contains the whole information, and it is mirrored in the other strand: A is always facing a T, C is always facing a G. Three letters, a *triplet*, form a word, which can code for an aminoacid in the mature protein. In the cell nucleus, DNA has to be *transcribed* into messenger RNA (mRNA; ribo-nuclein-acid). mRNA has to be processed (the ends are often cleaved at the destination) and transported to ribosomes, where *translation* takes place. During translation, the ribosome takes the mRNA, reads it triplet for triplet and adds for each triplet a new aminoacid, which is provided by a transfer RNA (tRNA). Each aminoacid has its own type of tRNA, which binds almost specifically to the mRNA (there are 4x4x4=64 different words, but only 23 aminoacids, so some aminoacids have different codes; stopping a sequence has its own '*stop-code*').

1.1.2 Genes

The definition of genes is a matter of debate, but for our purposes it is sufficient to say that a gene is a chromosome sequence which encodes a certain protein. It consists of one or several *exons*, whose translation can be found directly in a protein (are *ex*pressed), and *introns*, the sequences between the exons. Introns are important in eucaryotic genes, because they can guide the expression of a gene or modify the produced proteins. In complex organisms like humans, every cell type needs a different set of proteins, and many cells develop and change their

requirements. To accomplish this, the cell needs sophisticated tools adapt the transcription to the individual cell. This is done with help of introns: *Transcription factors*, special proteins, can bind the *promoter* of a gene, an intronic region ‘upstream’ (which means ‘before’ in the direction of transcription) of the first exon, and regulate the transcription. Introns between exons can lead to ‘*alternative splicing*’ which means that certain exons are not translated and therefore are not built into the product, a mechanism which can also be regulated by enzymes.

1.1.3 Mutations

There are different kinds of mutations (Knippers, 1997): Single nucleotides or longer sequences can be deleted (cut out), inserted, translocated to another place or inversed. Deletion of just one nucleotide is fatal, because all letters in the whole sequence are shifted by one, and may change their meaning. Exchange of one nucleotide can have different kinds of effects: Possibly (often if the third letter of a triplet is exchanged) the triplet still codes for the same aminoacid. If the new triplet codes for an aminoacid with similar features, then the function of the protein may stay similar. If the new triplet codes for a very different aminoacid, the function of the protein can be changed or impaired (‘*missense* mutations’). Biochemists often try to simulate how a protein is changed by mutations in a certain place. Of course they can change the three-dimensional structure or the enzymatic activity of a protein. Or a triplet in the sequence is modified into a stop-code, then the production of the protein is stopped at that point, and the protein is shortened and may not be able to fulfill its function (‘*nonsense* mutations’).

1.1.4 Polymorphisms

If a mutation happens to be stable in a population, which means that its carriers are able to confer it to their offspring, then it is called a polymorphism. If a polymorphism consists of an exchange of only one letter, it is called a single nucleotide polymorphism (SNP).

1.1.5 Chromosomes and Meiosis

The human DNA is stored in two times 23 chromosomes: 23 from the mother, and 23 from the father. A *chromosome* is an X-shaped DNA molecule consisting of two *chromatides*, which are linked near the middle. The DNA is wrapped on certain molecules, the histons, which can be regulated to extend or hide certain parts of the chromosomes. The chromosomes are numbered according to their length, so number 1 is the longest chromosome (different chromosomes of each type are called *homologous* chromosomes). Since the mother has also two times 23 chromosomes, and the father as well, our genome is a selection of 50% of the genome of the father, and 50% of the genome of the mother. Mother and father provide one set of 23 chromosomes each, which is a selection of the genome of their parents. During the '*meiosis*' the DNA from the grandparents is mixed (Knippers, 1997, page 210f): First, there is *homologous recombination*, which can exchange pieces of one chromosome with the homologous piece of another chromosome. Second, only one homologous chromosome (from the grandfather or the grandmother) is assigned to each gamete (sperm or ovum). Both mechanisms together act like shuffling cards: They mix and select genes. Nearby genes are likely to be inherited together. During fertilization, the motherly 23 chromosomes and the fatherly 23 chromosomes are fused in one cell.

1.1.6 Regulation

The reader may have noticed that several steps from the DNA to the protein can be regulated: Histons can make parts of chromosomes more or less accessible. Transcription factors can bind to intronic DNA and promote the transcription or lead to alternative splicing. The *RNA* is processed and has to be tagged to find its right place in the cell to be translated by ribosomes. mRNA is short-lived, and its turnover (its lifetime) can be regulated by certain mechanisms. Some genes do not code for proteins, but for regulatory RNA, which can interfere with transcription or translation. In addition, the DNA itself is processed by adding methyl groups. So we have to refine the idea of the genome as a book that is read from the beginning to the end. We should see the genome rather as a toolbox, where certain mechanics like certain

tools and even modify them for their purposes. This is the most basic level where environment already starts to *work with* the genes.

1.2 Memory

A definition of memory from a psychological point of view can be kept quite simple: Memory is each behavioral change that is influenced by experience. This very broad definition can be narrowed by two kinds of constraints: For example, psychologists have defined memory only as those behavioral changes that depend on the central nervous system. The problem with this constraint is that often it is impossible to verify if the central nervous system is involved (and probably it is), and no one knows where the memories are stored. The second kind of constraints is the level of consciousness of the retrieved information. The problem with this kind of constraints is that consciousness is difficult to measure, first of all in animals. We will have to discuss this second constraint (chapter 1.2.2 about memory systems).
For the observation of memory, two situations are important: The *encoding* situation, and the *retrieval* situation. In the encoding situation, information of some form is presented, and in the retrieval situation, the observed subject shows some form of use of the information encountered in the encoding situation.
Theorists have introduced a hypothetical process that should happen between encoding and retrieval: *Consolidation*. Consolidation describes a process of progressive stabilization of memory after acquisition (see Dudai, 2004 for a good review). Consolidation can be seen from two different perspectives: As a ‘hardening’ of memory traces (cellular perspective) or as a restructuring into a form that fits better into the system and therefore can be stored better (systems perspective).
There is a term that describes the contrary from a systems perspective: *Interference*. Interference is impaired retrieval by encoding of similar materials. Interference can be circumvented by storing additional information that makes the stored items less similar (for example, try to recall what you ate for lunch yesterday, the day before yesterday and so on; it will be easier to recall if the circumstances were very different each time than if you ate at the same place with the same people each time).

We will separately discuss the cellular level and the systems level of memory- both will be important for understanding the present work.

1.2.1 Cellular Level

1.2.1.1 Hebbian Learning and LTP

Hebb (1949) proposed a very simple mechanism for cellular learning: If a cell A is able to activate a cell B, and A activates B several times in a row, then it will become easier for cell A to activate cell B. Or to reformulate it in the language of neurophysiology, repeated activation of an excitatory *presynaptic* neuron can strengthen the synapse, so activation of the *postsynaptic* neuron by the presynaptic neuron is more probable (the term *synapse* is very important: a synapse is a small slit between two neurons, which enables neurotransmitters to proceed from the presynaptic neuron to the postsynaptic neuron, which produces voltage increases or decreases in the postsynaptic cell; see Figure 1).

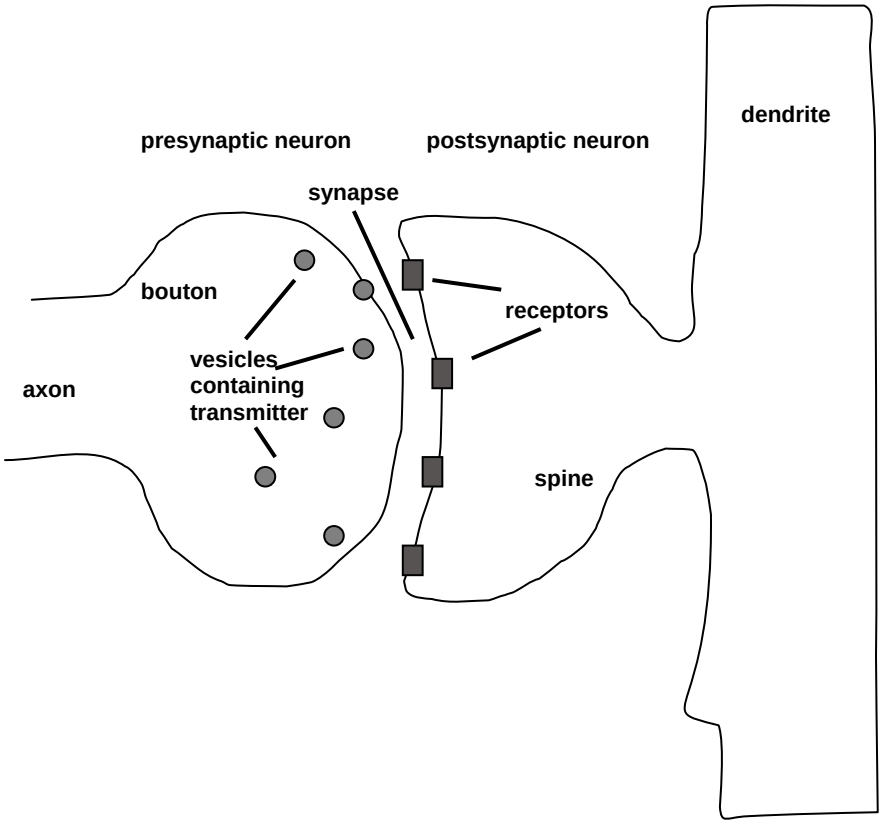

Figure 1: a typical synapse

This '*Hebbian Learning*' rule has been built into numerous learning models in neural networks. Such a mechanism was found some years later in the hippocampus of rabbits (Bliss and Lømo, 1973): *Long term potentiation* (LTP). They stimulated the perforant path in anaesthetised rabbits with high-frequency stimuli and found that this intervention lead to a stronger *excitatory post-synaptic potential* (EPSP: a positivation of the voltage in the postsynaptic neuron) reaction to single pulses for a time span of hours to days. The inverted effect does also exist: Lowering the tonic activity of the presynaptic neuron can decrease the reaction to single stimuli in the postsynaptic neuron (*long term depression*: LTD).
Kandel (2001) splits LTP in two phases: *Early LTP* does not depend on new protein synthesis, starts immediately after delivery of the high-frequency stimulus by the presynaptic neuron and lasts 1-3 hours at longest. *Late LTP* does depend on new protein synthesis, starts some minutes after the high frequency stimulus and lasts for at least one day. Figure 1 shows the involved mechanisms schematically.

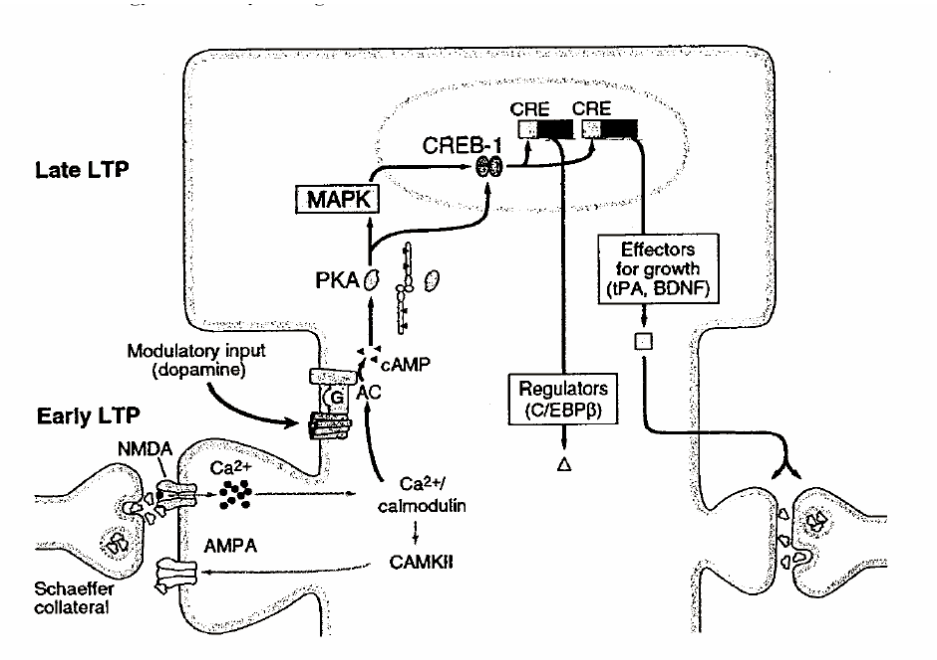

Figure 2: Early LTP and late LTP mechanisms (from Kandel, 2001; explanations in the text)

Early LTP works as follows: Repeated stimulation of the cell with the neuronal transmitter molecule glutamate leads to a strong depolarization of the postsynaptic cell (the big cell shown in Figure 2). Depolarization means, that the cell, which has normally a negative electric potential compared to the intracellular space, gets more positively charged. This process can open NMDA channels, which leads to higher calcium (Ca^{2+}) influx. The calcium activates calmodulin-kinases CaMKII and CaMKIV, which increase the probability of AMPA channels to open (Miyamoto, 2006). In parallel the activated calmodulin activates an enzyme called adenylylcyclase (AC). This leads to a reaction which splits ATP (adenosine triphosphate) into cyclic adenosine monophosphate (cAMP). cAMP has an influence of potassium (K+)-channels, which close earlier. This leads to a stronger electric pulse (*action potential*) moving down the axon, so the Ca^{2+}-channels at the synaptic bouton can open longer and more transmitter is released into the synapse, which leads to a stronger EPSP in the postsynaptic neuron. Monoamines can influence this process: Dopaminergic D1- and adrenergic β-receptors activate AC, while the serotonergic $5HT_{1A}$-receptor inhibits AC (Izquierdo et al., 2006).

Late LTP works as follows: cAMP activates protein kinases like PKA (protein kinase A) and MAPK. Irvine (2006) states that while in neonatal mice, PKA is the most

crucial messenger, CaMKII is more important in adult mice. CaMKII activates numerous important proteins like PSD-95, tubulin and cAMP phosphodiesterase (Izquierdo et al., 2006).
PKA, CaMKII and ERK (extracellular signal-regulated kinase) activate the CREB-protein (cyclic adenosine monophosphate responsive element binding protein)(Izquierdo et al., 2006). CREB is a transcription factor (see chapter 1.1) which binds to a promoter (CRE) and increases transcription of the following genes into mRNA, which finally leads to higher concentrations of certain proteins in the cell. These proteins can increase the efficiency of synapses in different ways: For example, the cell produces more AMPA receptors, which increases the efficiency of the transmitter glutamate. The cell also expresses retrograde messengers (like nitric oxide, NO, or brain-derived neurotrophic factor, BDNF, see chapter 1.4.4), which are brought to the presynaptic cell and trigger changes that ultimately lead to more transmitter release per presynaptic pulse, but also to growth of the synaptic machinery like axonal boutons. The changes on both sides of the synapse have to be coordinated. This works probably over cell adhesion molecules like the cadherins or NCAM (Lüscher et al., 2000). Synaptic elements like transmitter receptors are tied to the cytoskeleton, which consists of microtubules.
Izquierdo and colleagues (2006) point out that there are regional differences in the brain of which of these messengers are involved. However only the hippocampal CA1-structure and the amygdala have been investigated extensively.

1.2.1.2 LTP and Spatial Memory

It has been believed for a long time that LTP in the hippocampus is the physiological correlate of spatial memory in rodents and episodic memory in humans (see chapter 1.2.2). A direct proof for this has been presented only some weeks ago by Whitlock and colleagues (2006). They used two criteria to show the relation between memory and LTP: *mimicry* and *occlusion*. First they found that learning in an inhibitory avoidance task in rodents has the same effect in the dorsal part of the hippocampal subfield CA1 than high-frequency stimulation of the Schaffer's Collaterals (mimicry). Subunits of glutamatergic AMPA receptors were phosphorylated in the same place, a mechanism which strengthens signal transmission in the synapse. In addition, there

were more AMPA-receptor subunits found in the synaptoneurosome fraction. Third, the field excitatory postsynaptic potential (fEPSP;) was increased in CA1. In the places where these changes occurred, high-frequency stimulation had less effect on these three variables (occlusion) than in places with a smaller learning effect. This ingenious study leaves open a small gap in showing that the two processes, memory formation and LTP, are perfectly parallel: It does not show an influence of changes in LTP on memory formation. This does a second study in the same issue of Science (Pastalkova et al., 2006): The authors succeeded in showing that ZIP (ζ pseudo-substrate inhibitory peptide) an inhibitor of PKM ζ (protein kinase M zeta), a factor which is necessary and sufficient for LTP maintenance, practically abolished retention in an active avoidance task, where the rat has to avoid a fixed sector in a rotating field to avoid an electric shock. There was no effect of ZIP on memory acquisition or short term memory.

1.2.1.3 Some Basic Forms of Memory

Investigating memory in complex animals like rats is very difficult: There are several millions of neurons involved in the simplest processes, and neurons are very small and densely packed. Therefore Kandel (2001) and his coworkers followed the strategy to use a very simple model organism, the sea snail aplysia, which has only about 20000 neurons, the functions of which are relatively easy to find. In the beginning no one believed that something complicated like memory would be represented in a simple mollusk like *aplysia*, but Kandel and colleagues found some basic mechanisms that are very helpful to understand more complex forms of memory. They proceeded in a highly systematical manner and first isolated a reflex that can be modified by learning, then looked for the neural circuits involved and then for the molecular mechanisms. Kandel and coworkers (reviewed in Kandel, 2001) chose a simple defensive reflex of aplysia: Whenever the siphon (a mantle cavity around the gill) is touched, aplysia withdraws the gill. Kandel and colleagues described three processes which we know from psychophysiology and behaviorist

psychology: Habituation, sensitization and classical conditioning. All of these processes could be observed in the same synapse. There were two forms of memory in all of these processes: A transient form, which lasts minutes ('short term memory'), and an enduring form, which lasts days ('long term memory'; note that these concepts are not the same as the psychological terms, which are systems-based and have a different time scale). Long term memory could be induced by stimulating four or five times in a timely spaced manner.

Habituation (weakening of the response by repeated, often regularly spaced inputs) led to a homosynaptic (only 1 synapse involved) weakening of the synaptic connection. *Sensitization*, in contrast, led to a heterosynaptic strengthening of the synapse: A third, modulatory neuron gave input to the postsynaptic neuron, which then showed a stronger reaction to a pulse of the presynaptic neuron. Sensitization had also to do with a stronger excitability of the presynaptic neuron (Figure 3).

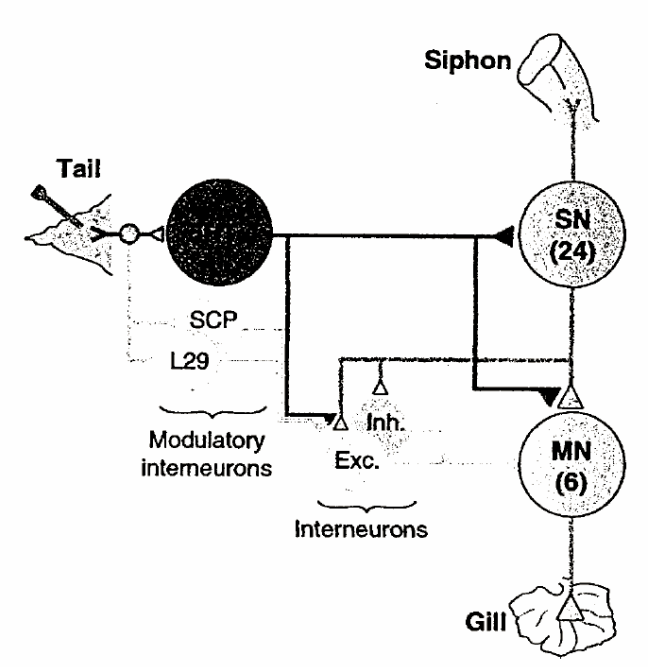

Figure 3: Sensitization in aplysia (from Kandel, 2001): SN= sensory neuron, MN= motor neuron, black neuron= modulating neuron leading to sensitization

Later they found out that the modulatory neurons work with the monoamine transmitters, which are also present in human brains: serotonin (5HT) and dopamine (DA). Kandel notes that this mechanism of heterosynaptic sensitization by serotonin could be interpreted as an attentional mechanism.

1.2.1.4 Synaptic Tagging

Kandel and his coworkers solved another very important problem with aplysia. For a long time it was not understood how learning can take place at the level of a single synapse: How can one synapse alone change its synaptic strength (weight) without changing the weights of all other synapses of the same axon? If rising cAMP levels increase the production of plasticity-related protein like AMPA-receptors in the core, then those products are transported to all synapses of the same axon.
Kandel and coworkers found out that synaptic boutons can be tagged with serotonin. One puff of serotonin was enough to mark a synapse, so it was the only synapse which was able to use the proteins coming from the cell nucleus if the cell was sensitizised. The mechanism behind this effect was that serotonin activated protein kinase A (PKA), which activated some growth factors, which promote mRNA stability and local translation of mRNA into proteins needed for the synaptic strengthening. If mRNA is around, this is a relatively fast process, because the synaptic endings contain ribosomes, which are the cellular translation machinery.

1.2.2 Systems Level

In chapter 1.2.1 we discussed memory of two or three single cells. Kandel (2001) showed that even the simplest monosynaptic reflex can be modified by experience in several different ways. Memory seems to be a very general feature of virtually every cell in the central nervous system; there is even short term memory and long term memory, but in another sense than in the psychological literature. There we have to deal with different systems. While behavioristic learning psychologists proposed that it is not crucial to know the processes that mediate between stimulus and response, cognitive psychologists chose an ‘engineer-like’ account figured out how you would build a memory if you had to construct one, and worked with computer analogies. What I mean with an ‘engineer-like’ account, can be explained with the assumption “in a reasonable system, for every function there is a device which fulfills that function”. This is the way how an engineer would construct a human being.

Experimental psychologists worked with *systems* for years. This is reasonable if you are just interested in functions, but not in how these functions are implemented in the organism. Only relatively recently psychologist began to worry about “if you can find a memory in the brain” and to learn methods to measure it there. This was the birth of neuropsychology. In a way, it is naive to think that we could find something like “a memory system” in the brain, since nature does not care about psychologist concepts. Keep this in mind when we discuss “physiologically plausible” models of memory. The great challenge about neuropsychology is to find ‘bridge’ concepts that make sense in both physiology and psychology. But in a way, the human mind is very stubborn: If you once integrated a concept, it is very difficult to describe the same phenomenon with new concepts. So we should be cautious with parallels between physiologic observations and psychological concepts.

1.2.2.1 Memory Systems

Squire (1987) differentiated *declarative* memory from *nondeclarative* (e.g. procedural memory, priming; see Figure 4).

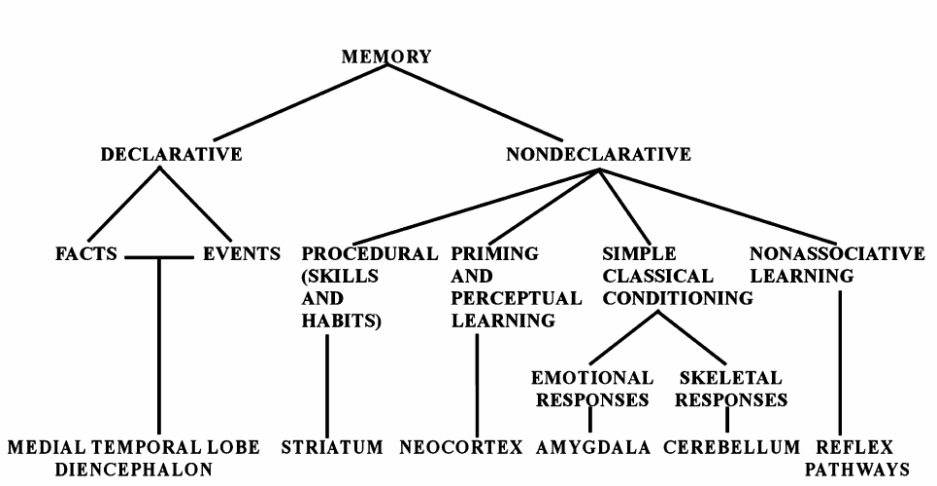

Figure 4: Memory systems according to Squire (2004)

The graphic assumes that there are two fundamentally different categories of memory: The learned things you may be able to declare if you are asked (declarative), and the learned things you may not be able to explain if you are asked

(nondeclarative). There is a second assumption suggested in the graphic: There are different brain areas that can be assigned to declarative versus nondeclarative memory. At the first glance, this looks simple and quite intuitive in an ‘engineer-like’ manner: Every function has its brain area.

Tulving (1972) further differentiated declarative memory into *episodic* and *semantic* memory. While episodic memory involves contextual knowledge (about time and space something was learned in; recollection), semantic memory does not involve contextual knowledge, but bases on familiarity. Tulving called episodic-like answers in a memory-test ‘Rembember’-answers, semantic-like answers ‘Know’ (Tulving, 1985). This refines the issue about conscious and nonconscious memory content: The same item is remembered in a more conscious manner, if you recollect also where and when you encountered that item. The same item can be primed and then consciously remembered. That declarative and non-declarative memory systems cross-talk has been shown by at least two studies:

An interfering effect of priming on subsequent episodic memory retrieval was shown by Wagner and coworkers (Wagner et al, 2000). This held true for both behavioural priming and correlated repetition suppression in memory related brain areas as the left inferior prefrontal cortex (LIPC: BA 47, 45, 44 and 6) and the posterior fusiform gyrus (BA 37): Stronger priming at encoding resulted in reduced episodic memory retrieval performance. The authors mention that this is a system-based explanation for the spacing-effect already observed by Ebbinghaus (1885). As a mechanism, they propose reduced encoding variability: The task-related attention of certain features of a stimulus could reduce the number of ways the stimulus can be retrieved (Wiggs and Martin, 1998). In a study with subliminal presentation of face-profession pairs, Degonda and coworkers (Degonda et al., 2005) showed a negative influence of unconscious semantic associative priming on subsequent episodic retrieval. Interesting is the pattern of correlations between hippocampal activation at unconscious encounter of the stimuli and subsequent retrieval performance: If the primed profession associated with a face was incongruent with the profession subjects had to associate with the face afterwards, higher hippocampal activation went with higher retrieval performance in episodic memory; if the primed profession was congruent (same professional category) or identical with the profession learned in the subsequent episodic task, hippocampal activation went with lower retrieval

performance in episodic retrieval. This pattern speaks for a reversal of the beneficial influence of hippocampal activity in episodic memory.

1.2.3 Memory Structures

1.2.3.1 The Hippocampus

Some general features:

The *hippocampus* is the most prominent structure of the medial temporal lobe. It has an ideal place to connect lateral temporal areas with parietal and prefrontal areas. It is tightly connected to the parahippocampal cortex and receives its principal inputs from there. Output structures are also the parahippocampal cortex (over the subiculum), but also nuclei in the anterior thalamus (over the fornix fibers) and the hypothalamus (corpora mamillaria). Anatomical observations lead to the notion of a simple feed-forward structure, which should be organised in lamellae, so that each point in the parahippocampal cortex projects within the same y-coordinate (anterior-posterior) into the dentate gyrus, from there to the CA3 subfield, then to the CA1 subfield, and over the subiculum back to the parahippocampal cortex (Figure 5).

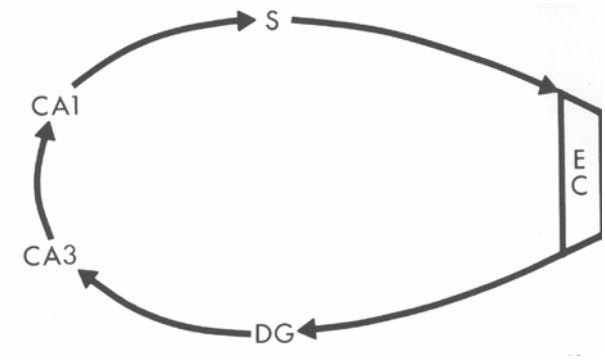

Figure 5: Organisation of the hippocampus (from Amaral & Witter, 1989)
EC: Entorhinal Cortex (a part of the parahippocampal cortex); DG: Dentate Gyrus; CA1/3: Cornu Ammonis; S: Subiculum

Amaral and Witter (1989) criticized this simplicistic view and stressed the fact that the antero-posterior dimension is also important: For example autoassociative fibers from the dentate gyrus move over considerable distances along the antero-posterior axis. Besides, the simple feedforward structure is too simple and has to be replaced by a more complicated model (Figure 6).

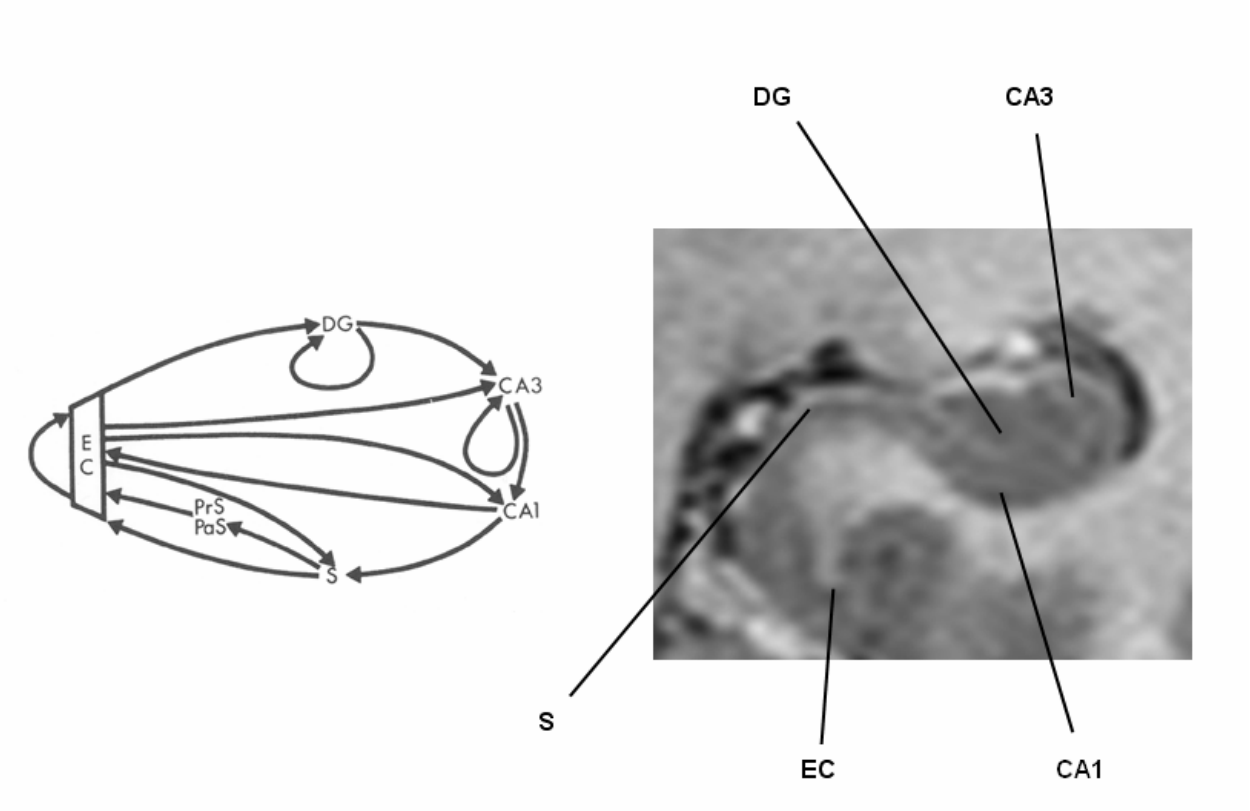

Figure 6: More sophisticated model of hippocampal organisation (left; from Amaral & Witter, 1989) EC: Entorhinal Cortex (a part of the parahippocampal cortex); DG: Dentate Gyrus; CA1/3: Cornu Ammonis; S: Subiculum; PrS: Presubiculum; PaS: Parasubiculum

Physiology:

It has been shown by several authors that the hippocampus shows a slow, large amplitude, almost sinoidal activity, which is probably triggered by pacemaker cells located in the medial septal nucleus and the diagonal band of Broca (‘theta’ waves). It has been shown that administration of sodium amobarbital raises the threshold of hippocampal theta after artificial stimulation in the medial septum, especially at the frequency produced spontaneously during exploration (Gray & Ball, 1970), which

could explain the behavioral effects of amobarbital on extinction learning. The pathway from the medial septum over the ventral or dorsal fornix to the hippocampus is more or less paralleled by cholinergic (by acetylcholine; ACh) activity; transsection abolishes theta (Rawlins et al., 1979). Theta can be triggered by stimulation in the midbrain (see e.g. Pavlides et al, 1988). These authors have shown that induction of LTP in the perforant path is modulated by theta: With tetanic stimuli administered near the peaks of theta, the threshold is significantly lower than in the troughs. While theta waves are observed mainly during exploratory behaviour, in the rest of the time (during consummatory behaviour, immobility and slow wave sleep) there can be hippocampal sharp waves ('SPW') in CA3, often timely adjacent with high-frequent (about 200Hz) 'ripple' activity in CA1. These ripples are a population pattern of several pyramidal neurons, which fire only in a small percentage of the peaks, but in a synchronised manner, which is probably imposed by highly synchronised inhibitory interneurons, which impose the frequency structure on the firing (Ylinen et al., 1995).

Classical theories of hippocampal function

O'Keefe and Dostrowski (1971) implanted electrodes in rats and found cells in the hippocampus that responded only if the rat was on a certain place on the experimental board and faced a certain direction. The reaction was more broadly tuned (less selective) if the animal was aroused. The authors suspected that these cells act as a spatial map and provide the rat with room-centered information ("cognitive map theory"; CM).

Olton and colleagues (1979) contradicted to the hippocampus processing spatial maps and proposed a 'working memory theory'. They defined working memory as dealing with information that changes from trial to trial, in contrast to reference memory, which deals with information unchanged over trials, like general rules. They showed in an elegant experiment with a 17-arm maze that rats with hippocampal lesions are impaired in working memory, but not in reference memory: Arms never baited were not searched by the lesioned rats, but they did not know which of the baited arms they had searched before. Olton and colleagues pointed out that O'Keefe's results speak also for this theory, since there were "displace" nerve units that responded to changes in stimulus arrangements from visit to visit. Note that the term 'working memory' here has a completely different meaning than in the human memory literature. Olton and colleagues pointed out that though a working definition,

‘working memory’ and ‘reference memory’ nicely parallel ‘episodic memory’ and ‘semantic memory’, as used by Tulving (1972). Olton’s theory can be seen as part of multiple trace theory (see below).

Solomon (1979) concluded from experiments with rabbits (like latent inhibition: in this paradigm, animals are preexposed to the to-be-conditioned stimulus, which inhibits later classical conditioning) that the hippocampus is not only involved in learning spatial maps, but also ‘temporal maps’, i.e. temporal sequences of stimuli. Authors agree that simple cue learning does not need hippocampal function.

Rawlins (1985) proposed a ‘discontiguity theory’ of hippocampal function. This theory stresses the fact that hippocampal-lesioned animals often do well if they have to associate events or objects which overlap in time (e.g. classical conditioning), while they fail to associate events or objects that are separated in time more than some seconds (e.g. trace conditioning).

Teyler and DiScenna (1986) proposed a “hippocampal memory indexing theory”. This theory asserts that the distributed assemblies of cortical modules activated by a new event are stored in the hippocampus.

Alvarez and Squire (1994) proposed a model to explain system consolidation between the medial temporal lobe (MTL) and the neocortex. In their view, the MTL is a fast-learning, limited-capacity store. For each learned event, a unique network binds widely distributed cortical sites. This network can reinstate the whole representation if it is fed with a cue. During consolidation, the cortex grows gradually more able to execute this function without help of the MTL, so the MTL can recycle its limited capacity for new events (Figure 7). The cortical store, in contrast, is slow-learning and has a practically unlimited capacity.

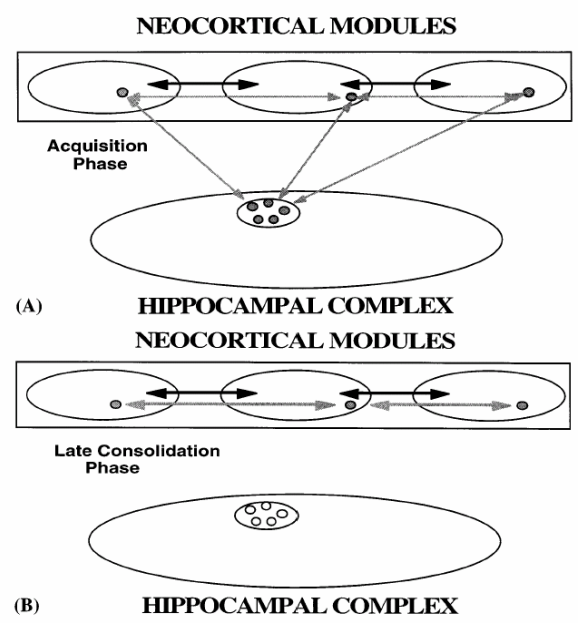

Figure 7: The Alvarez & Squire (1994) (“SC”) model of declarative memory consolidation. From Nadel & Moscovitch, (1998)

Nadel and Moscovitch (1998) proposed a different model of declarative memory function and consolidation: They noticed that the Alvarez and Squire model has to assume a maximum consolidation time window of up to 20 years or more to explain memory deficits in amnesics. Nadel and Moscovitch pointed out that it is sparser to assume that the hippocampal system is involved not only in episodic encoding, but also in episodic retrieval, in line with evidence of imaging studies in human (e.g. Rekkas & Constable, 2005).

Their model is connectionistic and in line with a constructivistic view of thinking and memory: An event consists of lots of different aspects, which are probable to be stored in different cortical regions. The hippocampal system accomplishes the binding of all these aspects into an event in its temporal and spatial context. The authors point out that episodic memory in humans matures at an age of 18 months at earliest, at the same time as orienting with help of spatial maps. Before that age or in amnesics, there is just semantic knowledge (facts without temporal or spatial context). The context, which means which aspects belong to an event, is stored in the hippocampal system exclusively. This is why there is no *episodic* retrieval without a functional hippocampal system. Retrieval can be initiated from both contextual nodes (i.e. from the hippocampus) or from aspect nodes (i.e. neocortex). The theory

also states that episodes can be semantisized and become independent of the hippocampus, but then they change their form and are less vivid and flexible (Moscovitch et al., 2006). Moscovitch and colleagues stress the point that the *quality* of memory traces (vividness, emotionality, personal significance) has to be taken into account: Probably retrieval of vivid, episodic representations (recollection instead of familiarity; see Tulving, 1972) stay hippocampus-dependent. An interesting side-issue is that episodic and semantic knowledge have parallels in the theory of concepts: Semantic knowledge would be on the level of prototypes, episodic knowledge on the level of exemplars (Nadel & Moscovitch, 1998).
The issue of retrograde amnesia in terms of this model is explained with multiple contextual traces in the hippocampal system (this is why this model is called the "multiple trace model", see Figure 8). With each recall, there is a new trace built at a random place in the hippocampal system. These traces bind similar but not identical nodes of the neocortex. In case of hippocampal damage, elder memories have more contextual traces and are therefore less likely to be erased, while new memories with less traces are likely to be erased. The model explains why in extensive hippocampal damage, the amnesia for semantic knowledge can be graded, while the amnesia for episodic knowledge is total.

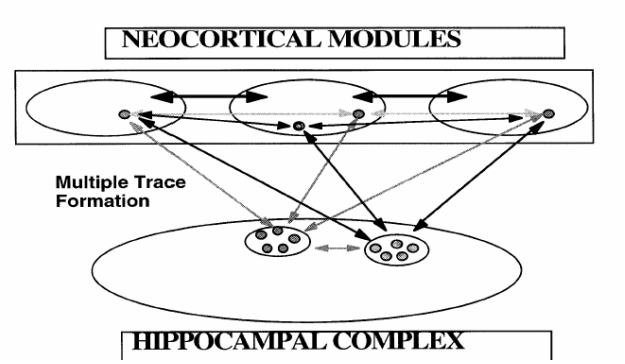

Figure 8: The multiple trace model (MTT) for episodic memory (Nadel & Moscovitch, 1998)

While practically every listed theory explains one aspect of hippocampal function very well, they do not explain hippocampal function as a whole. The cognitive map theory explains the very interesting findings in animals, while Squire's system consolidation theory is rather based on observations of human amnesics. The most modern and plausible theory is the multiple trace theory, though it has also a limited focus. What

is the common element between spatial learning (that correlates best with hippocampal function in animals) and episodic learning? The simplest explanation might be that binding of several objects into an event needs data from different modalities, which are stored in the parietal lobe (place) and temporal lobe (objects). Also the aspect of time seems to be important. What the hippocampus probably does is fast one-trial association of objects belonging to one episode in the temporal order of episodes.

Subregions:

Some studies suggest differences along an anterior-posterior axis: In a study investigating true and false memories for words, Cabeza and colleagues (2001) found that activations in the anterior hippocampus correlated rather with the subjective experience of memory (false memories along with true memories), while more posterior activations in the parahippocampal cortex separated true memories from unstudied items. A similar dissociation was found in a second study (Daselaar et al., 2006).

Ideas about hippocampal function from newer studies:

There is an ongoing debate of in which types of memory and which processes the hippocampus proper or the hippocampal system is engaged. Lesion studies in animals seem to show that lesion of only the hippocampus seems to have small effects. In humans, lesion of both hippocampi leads to severe impairment of the whole episodic memory. However, it seems to be difficult to find hippocampal activation in imaging studies. Possibly the subtraction technique used in fMRI is not enough sensitive to hippocampal activation, because the hippocampus is similarly active in baseline tasks (Stark & Squire, 2001). Another explanation for the discrepancies between human lesion and imaging studies are differences in the kinds of representation: For example, dealing with very familiar materials like words could diminish hippocampal activation because of the ‘semantic-like’ character of the task. Related to this explanation, the hippocampus could serve a kind of association device (Ungerleider, 1995) or serving in tasks that require flexible, relational representations (Cohen & Eichenbaum, 1993). In a similar vein, Morris (1996)

concluded from spatial learning studies with NMDA-receptor-blocked rats in two different environments, that spatial learning per se is possible without hippocampal LTP, but strategies learned in the first environment can no longer be used in the second environment. Morris concludes that the function of the hippocampal system is the temporary registration of episodic information in one-trial into an abstract representation.

Memory activity of the medial temporal lobe (which can not be measured more directly with EEG) and wider parts of the brain are also reflected in electrophysiological measures, in terms of activity in the theta- and gamma- range. In a study with electrocorticograms ('internal EEG' or 'iEEG') in epileptics, Sederberg and colleagues (2003) found increased theta activity in the right temporal/frontal cortex in successfully learned words than in forgotten words. A second study found similar results with MEG in a picture task in healthy subjects: During subsequently remembered items relative to forgotten items, there was increased theta activity over right parietotemporal areas, while gamma activity was found over BA 18/19.
Fell and colleagues (2001) could show altered rhinal-hippocampal coupling during successful encoding of words compared to unsuccessful encoding: Synchronicity was first higher, than lower in the course of successful encoding. The synchronisation started 200 ms after stimulus onset, the desynchronisation at 1000-1100 ms after stimulus onset. The authors suggest that the synchronisation starts before semantic information has reached the medial temporal lobe, while the 'active desynchronisation' after 1000ms is a sign of successfully exchanged semantic information.

Electrophysiological studies are beginning to monitor dynamic changes while an animal is learning something, like exploring a new radial maze: Frank and colleagues (2006) compared the activity of hippocampal CA1 place cells with entorhinal cortical cells. They found that the hippocampal cells showed a very rapid plasticity upon exposure to a new environment, and instability of the representations formed at the beginning of the exploration. There were some CA1 cells that started to be place cells only during exploration, while most of the cells were place sensitive already in the beginning. In contrast, the cortical cells changed their firing patterns more slowly, were not very place sensitive and showed a slow activity decline with training, but not as large changes as the hippocampal cells. They interpret their findings in terms of a

specialisation of the hippocampus to map rapid changes, whereas the cortex reacts much more slowly. This field of research is very interesting but quite at its beginning, since we are far from understanding the dynamic interactions of all the involved regions and sorts of cells.
It has been shown that the amplitude of the hippocampal theta during exploration is linearly dependent on the running speed of a rat (McNaughton et al., 2006). It has been shown that place fields grow larger if the animal is passively moved around, so place cells seem to depend on motor input (but possibly also on vestibular activation and optic flow).

1.2.3.2 Entorhinal/ Rhinal Cortex

Many papers ascribe the entorhinal cortex a prominent role in familiarity-based recognition. For example, Brown and Xiang (1998) review evidence that cells in the perirhinal system show a reduced reaction to repeated stimuli. In addition, Fernández and Tendolkar (2006) propose a second function of the rhinal cortex based on fMRI and ERP results: The rhinal cortex could serve as a 'gatekeeper' that reacts fast on the familiarity of a stimulus and gates encoding of too familiar stimuli. As a basis of their proposal, they mention a feature of the rhinal cortex, that can be found in both fMRI studies ('repetition suppression') and ERP studies ('anterior medial temporal lobe N400' or 'AMTL-N400'): This region activates less upon repeated encounter of a stimulus, while higher activation at encoding predicts a higher retrieval probability (there are also other brain regions showing this feature: The left inferior prefrontal cortex (Wagner et al., 2000)).
In the rat it has been shown that the entorhinal cortex close to the postrhinal-entorhinal border (near the output into the dentate gyrus) has place representations in the form of multicentric place fields (Fyhn et al., 2004) which transform into monocentric place fields in the hippocampus. Probably the hippocampal place fields depend on collective firing of several entorhinal multicentric 'grid' cells. This increases the stability of hippocampal place fields relative to entorhinal place fields.

In another study, the authors found out which entorhinal cell layers respond to which kind of stimuli (Sargolini et al., 2006): Grid cells are found in all principal layers (II through V). Some cells in layers III / IV, but not in layer II, respond to head direction (sometimes the same cells; “conjunctive” features). The firing of most cells correlated also with the speed of the rat. The authors hypothesize that the position vector of the rat in allocentric space is updated with this kind of information. The influence of the entorhinal cortex for spatial orienting is shown by studies with lesioned rats, which are not able to return directly to their home cage (reviewed in McNaughton et al., 2006). Possible grid cells are not formed by experience, but by neonatal cortical waves (McNaughton et al., 2006).

1.2.3.3 Extended Hippocampal System

In a review paper, Aggleton and Brown (2006) try to elucidate the importance of two diencephalic systems for episodic memory, the medial and the lateral system. The *medial* system comprises the subiculum, the medial mamillary nucleus, the anterior medial and anterior ventral thalamus and the ventral tegmental area. In this system there are neurons firing in the theta rhythm. The medial system could optimise encoding over feedbacks to the hippocampus and strengthen LTP. Via links to the frontal cortex it could serve strategic aspects of memory and reduce interference by separating between encoding and retrieval.

The *lateral* system comprises the presubiculum, the postsubiculum, the lateral mamillary nucleus, the anterior dorsal thalamus and the dorsal tegmental nucleus. In this system, there are head-direction cells (cells that fire only if the head looks in a certain direction), so it is implicated in navigation.

There are interesting ideas of information processing in the hippocampal system: Spatial and nonspatial information from the medial and lateral entorhinal cortex could converge on the hippocampus. An important role of the hippocampus in episodic memory could be the minimizing of similarities between representations (which is reflected in remapping of place cells on changing inputs, first of all in CA3, but also in CA1: Small changes lead to rate remapping, large changes of stimulus configurations

or context to global remapping, which is a complete reorganization of the hippocampal place code; McNaughton et al., 2006).

1.2.3.4 Amygdala

Gewirtz and Davis (1997) found a dependence of second order operant conditioning of NMDA receptors in the basolateral amygdala: Perfusion of an NMDA antagonist prevented efficient association of a second unconditioned stimulus (US) with a conditioned response. The intervention did not influence the already established first order conditioning. Similarly, Wallace and Rosen (2001) found that lesion of cells of the basolateral amygdala prevented contextual fear conditioning, but not unconditioned fear.
Henson and colleagues (1999) found that the (bilateral) amygdala activates more during 'New'-answers than during old ('Remember-' or 'Know-') answers in word recognition. They supposed that this structure does a kind of novelty detection for memory encoding, leading to a more effective encoding by other mediotemporal structures through arousal.

1.2.3.5 The Basal Ganglia

Packard and colleagues (1989) cite studies which say that the basal ganglia support simpler forms of memory (like habits or reference memory) whereas the hippocampus supports more sophisticated forms of memory (declarative memory, working memory in the "animal-researcher's" sense of the word; see Olton et al., 1979). In an own study, they found that hippocampal-lesioned rats were impaired in win-shift tasks (that require to know which arms of a maze have already been visited) but not in win-stay tasks (where cues show which arms are baited), whereas caudate-lesioned rats were impaired in win-stay tasks, but not in win-shift tasks. They observed that healthy rats have a tendency to win-shift if they have the choice between win-shift and win-stay. They conclude that the "hippocampal-memory system" and the "caudate-memory system" in this case compete in unlesioned rats. However, they note also that the importance of reinforcers is higher in the win-stay

task: They reinforce the reaction in a more direct manner. This led to the idea that the basal ganglia accomplish “cued learning” (e.g. Graybiel, 1995). This was elaborated with another study by Packard and McGaugh (1992), who were able to establish a double dissociation between caudate lesioned and fornix lesioned animals: Caudate lesioned animals failed in a version of the water maze task that depended on cues (one coloured ball was an escape platform, the other was not) and did well in a spatial version (the escape platform was always in the same quadrant of the maze), in fornix lesioned rats it was the other way round.

In a similar double-dissociation lesion study with rats, Kesner and colleagues (1993) found that while hippocampal-lesioned rats showed mainly impairment in a memory-task that required room-centered spatial navigation, caudate-lesioned rats were impaired in a memory-task requiring memory for responses in body-centered coordinates. They propose an ‘attribute memory model’ which states that which brain-regions are used for a memory task depends on the attributes of the stimuli that have to be learned.

A nice study in human patients (Knowlton et al., 1996) showed a double dissociation in a task where subjects had to predict good or bad weather with cards that mapped good weather probabilities: While amnesics solved the task, but did not remember the task episode, Parkinson’s patients remembered the episode, but were not able to solve the task.

In a study dealing with theoretical models, Atallah and colleagues (2004) propose a more sophisticated model which says that the role of the caudate is to support one reaction and to inhibit competing reactions (which are stored in the premotor cortex). The basal ganglia learn slower and therefore better fit the slow cortical learning. The model proposes that the ventral striatum receives more inputs from the hippocampus and the amygdala, so that lesions to the ventral striatum also impair spatial learning, while the dorsal striatum rather receives inputs directly from the posterior cortex, which impairs response-based strategies (Figure 9).

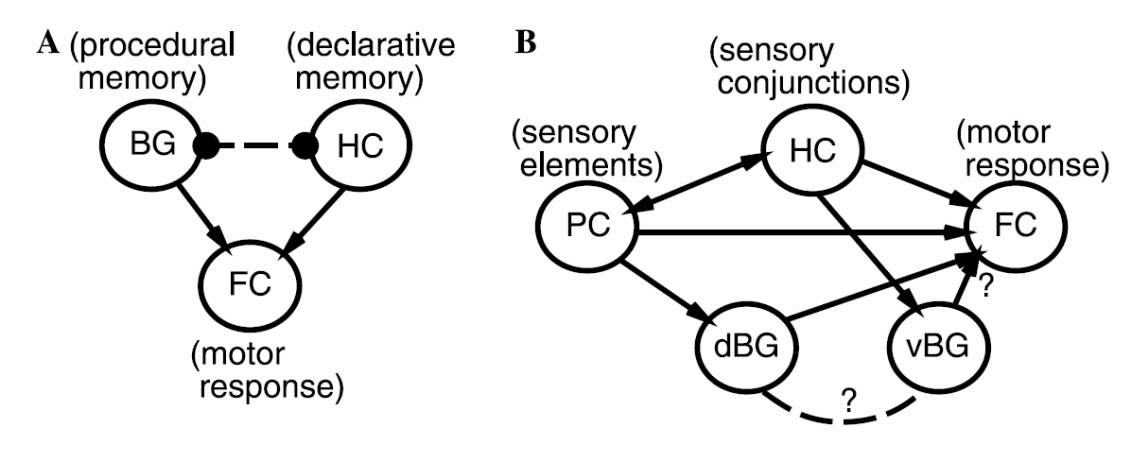

Figure 9: “Classical” and new model of hippocampal/striatal system interaction (from Atallah et al., 2004)

Atallah and colleagues propose another dichotomy important for basal ganglia function: The main effect of dopamine in the basal ganglia is to enhance “Go” firing or to inhibit “Nogo” firing. The authors say that “Go” cells express dopamine D1 receptors, while “Nogo” cells express D2 receptors, so dopamine in the caudate excitate “Go” cells and at the same time inhibit “Nogo” cells, which would explain the changes in “win-shift” or “win-stay” tasks. DA cells are known to activate upon unexpected reward and to dip if expected reward does not occur. A later paper (Frank, 2005) further elaborates these thoughts: The direct pathway from the striatum to the internal part of the globus pallidus (Figure 10) which promotes thalamocortical activation, could facilitate the execution of responses (“Go”), while the indirect pathway from the striatum over the external part of the globus pallidus to the internal part of the globus pallidus could inhibit the execution of responses (“Nogo”). Both kinds of inputs then could compete in the internal globus pallidus, where the most appropriate reaction (or stimulus provoking a reaction) could be selected and promoted.

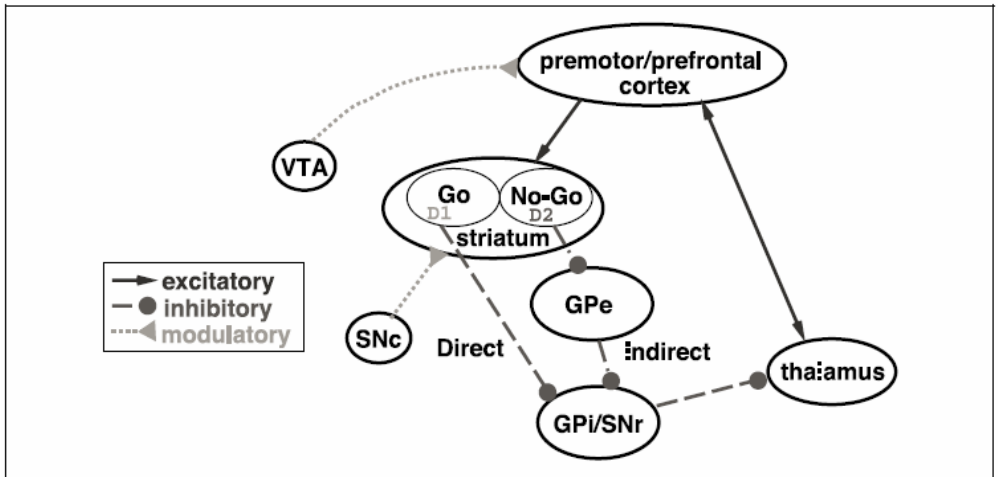

Figure 10: The direct and indirect striato-thalamic pathways (from Frank, 2005). VTA: ventral tegmental area; SNc: substantia nigra, pars compacta; SNr: substantia nigra, pars reticulata; GPe: globus pallidus, pars externa; GPi: globus pallidus, pars interna

The author cites literature stating that dopamine D1 receptor activation leads to sharpening of excitatory currents by enhancing signal-to-noise ratio (enhancing both excitatory and inhibitory effects of dopamine), while D2 activation inhibits excitatory currents. This should influence learning, since dopamine has been shown to support LTP. For example, Parkinson patients show impairment in 'probability reversal' task, where subjects have to learn with feedback that one event is more probable than the other, but in the middle of the experiment the probabilities switch, and the subject has to reverse his expectations without knowing the underlying rule. Medication enhancing dopamine transmission (L-Dopa or dopamine agonists) improve task-switching but impede this probability reversal task, probably by inhibiting unlearning of the probabilities of the first half in the second half of the experiment. fMRI studies show ventral striatum activation during error in the second half of this task. A more recent fMRI study showed caudate activation in trials with feedback as opposed to trials without feedback, and even a differential response in right caudate upon negative feedback compared to positive feedback (Tricomi et al., 2006). Frank concludes that the basal ganglia are critically involved in cognitive reinforcement learning. In addition, he proposes that dopaminergic activity in the basal ganglia could be related to the speed/ threshold of updating working memory content in the frontal lobe, which could explain the frontal deficit in Parkinson disease.

1.2.3.6 The Frontal Lobe

In an early PET study, Shallice and colleagues (1994) noted a hemisphere shift from encoding to retrieval: During encoding, the left prefrontal cortex was activated significantly in their study, during retrieval the right (they worked with different subjects during encoding and retrieval). Cabeza and Nyberg (2000) differentiated between brain regions involved in retrieval success (BA 10, 9 and 46 bilaterally), retrieval effort (BA 47 and 10, more left than right) and retrieval mode (BA 10 right). They mention a dissociation between free recall and cued recall: There are more activations in right BA 9 and 46 in free recall, but more activations of the frontal insula and BA 47 in cued recall.

Based on earlier studies and a study on true and false memories (see above), Cabeza and colleagues (2001) tried to classify frontal lobe contributions to memory into 3 subregions:

The *ventrolateral prefrontal cortex* (Brodmann area 45 in their study) is important for semantic processing and responds more to new and learned (old) items than to foils. The *dorsolateral prefrontal cortex* (BA 46, 9 and 8) seems to be associated with monitoring and responds more to learned items than to new and falsely remembered items. The *ventromedial prefrontal cortex* (BA 11) responds more during falsely remembered than old items and could reflect verification processes. Lesion of this region should lead to confabulations.

Rajah and McIntosh (2006) contrasted frontal brain regions activated in episodic memory retrieval (learning word pairs and their temporal order) with frontal regions used in a strategic control task (reversed alphabetizing of words). They found more activity in the left inferior frontal gyrus (BA 47) during episodic memory retrieval, but more activity in the right dorsolateral PFC during the strategic task (for example, right BA 9 and 44/ 45). The latter activation could be due to manipulation or monitoring.

Dove and colleagues (2006) contrasted *intentional* and *incidental* encoding and found bilateral mid-ventrolateral prefrontal (BA 45-47) activation in intentional encoding only. Intentionality correlated with retrieval success. They conclude that this region can reflect also intentionality in retrieval. They support a theoretical model which assumes that the ventrolateral prefrontal cortex biases modal representations in the more posterior brain over attentional processes.

Source Memory: ‘Source memory’ designates the ability to retrieve the sources of facts or events, i.e. when and where facts were encountered. Therefore, source memory can be seen as a special case of context memory. Studies with patients with frontal brain lesions often show impaired source memory despite normal memory for the events or facts (Janowsky et al., 1989). A side result of this paper is that source memory can diminish also in normal aging.
In an ERP study, remembering of the items could be seen in a P350 potential, while a later positivity 700-800ms after the stimulus could be seen over frontal areas, if subjects were asked for the source of the word (Senkfor and Van Petten, 1998). This potential was not sensitive to correct or false remembering of the source, so the authors conclude that it reflects the search for the source and not the successful finding of the source. They mention the possibility that search for an item or search for the source of an item could be similar processes, but source retrieval could often be more difficult, because it needs serial finding of two pieces of information. In a fMRI study, the authors tried to dissociate frontal areas involved in item recognition, source recognition and semantic encoding in a word task (Dobbins et al., 2002). The left posterior IPFC (Inferior PreFrontal Cortex; BA 44/ 46/ 9) was active in all of these operations; the left anterior IPFC (BA 45/47) was active during source recognition and semantic encoding; the left frontopolar (BA 10) and posterior dorsolateral PFC (BA 8) was exclusively engaged during source recognition. Activity in all these regions was not correlated with retrieval success, but probably more with retrieval attempt. In an ERP study, Kuo and Van Petten (2006) found an early (200-400ms) and a late (700-800ms; frontal) potential differentiating old from new items. Both potentials did not differentiate between correctly and incorrectly identified sources, but later (>800ms) posterior potentials did. Similarly to Dobbins and colleagues (2002) they conclude that prefrontal engagement has only to do with strategies to overcome weak memory traces, but not with source memory in itself.

1.2.3.7 The Anterior Cingulate

Anterior Cingulate activations (BA 24, 32) have been involved in response selection and initiation of action, but also in episodic memory retrieval, maybe more in verbal memory tests than with pictorial materials (Cabeza & Nyberg, 2000).

1.2.3.8 The Parietal Lobe

Cabeza and Nyberg (2000) summarize data stating that medial parieto-occipital regions are involved in episodic retrieval (mainly *retrieval success*), namely the retrosplenial cortex (BA 29 and 30), the precuneus (BA 7 and 31) and the cuneus (BA 19, 18, 17). Also lateral parietal regions seem to be involved in spatial memory and in the perceptual component of memory.
In the study of Cabeza and colleagues (2001), lateral (BA 39/40) and medial (BA 7) areae are implicated in recognition and respond to correctly remembered items more than to falsely remembered items (retrieval success).
Activation of these areas seems to be specific for episodic memory and not to be observed in semantic memory (reviewed in Ungerleider, 1995).

1.2.3.9 Occipito-Temporal Junction

Meltzer and Constable (2005) found a region at TT -51, -51, -17 (which corresponds to inferior temporal gyrus, BA37) which was active during both encoding and retrieval in a word pair association task.

1.2.3.10 Precuneus and Posterior Cingulate

There are numerous reports of precuneus activation during retrieval: for example, Shallice and colleagues (1994; word retrieval; TT -6 -68 36 and 12 -72 28); Fletcher and colleagues (1995; word retrieval; TT coordinates 6 -46 36, together with other activations in the bilateral precuneus, the left anterior cingulate and the right fusiform and superior temporal gyri). The authors conclude that the precuneus is involved in *imagery during retrieval*, and more speculatively, in conscious processes in memory retrieval ('visual imagery buffer' or 'mind's eye'). Henson and colleagues (1999) found that the left precuneus in a word recognition task shows a dependence on memory quality, in that 'Remember'-answers activate most, 'Know'-answers less, and 'New'-answers least.

A large review paper about the precuneus confirms this kind of view (Cavanna & Trimble, 2006): It ascribes functions with first-person perspective like self-consciousness and the general state of goal-directed behaviour (as opposed to sleep or anaesthesia) to the precuneus. It also seems to be involved in covert attention shifts (similar to the simultanagnosia observed in Balint's syndrome, where often only isolated parts of the field of view are perceived). The authors stress its high metabolic rate ('hot spot'). Besides, it has connections to practically everywhere (frontal BA 46, 8, 9, anterior cingulate, thalamus, basal ganglia, the 'temporoparietooccipital cortex') but NOT with primary sensory areas. They postulate that it can be separated in two functionally different areas, an anterior 'mental imagery strategy' area (about MNI +/- 10, -70, 50) and a posterior 'successful episodic memory retrieval' area (about MNI +/- 6, -67, 35; perhaps it would be more accurate to say 'dorsal' instead of 'anterior' and 'ventral' instead of 'posterior').

1.2.3.11 Summary

Looking at the broad range of brain areas that have been involved in memory, it becomes clear that memory is a matter of the whole brain. Roughly, we can separate the areas into two different parts: Areae that store information (like the lateral temporal lobe, wide parietal and occipital areas) and areae that integrate and select information (like the medial temporal lobe, basal ganglia, and frontal cortex). While activation of the first kind of areae often correlates with retrieval success, activation of

the second kind of areae can also have to do with difficulty in memory search (especially in the frontal lobe; retrieval attempt).

1.2.4 Memory and Sleep

Pavlides and Winson (1989) have shown a higher activity of CA1 place cells in both REM sleep and slow wave sleep (SWS) if exposed to their place during the day before sleeping relative to place cells not exposed to their place.
Wilson and McNaughton (1994) have shown that during *slow wave sleep (SWS)*, hippocampal place cells in rat CA1 encoding the same area in space coactivate if they have been coactivated during the day (Figure 11). These correlations seem to be stronger during hippocampal sharp waves. Sharp waves and ripples (a special form of bursts occurring during slow wave sleep; see above) are initiated in CA3. The coactivation during sleep is interpreted as ‘playback’ of learned episodes.

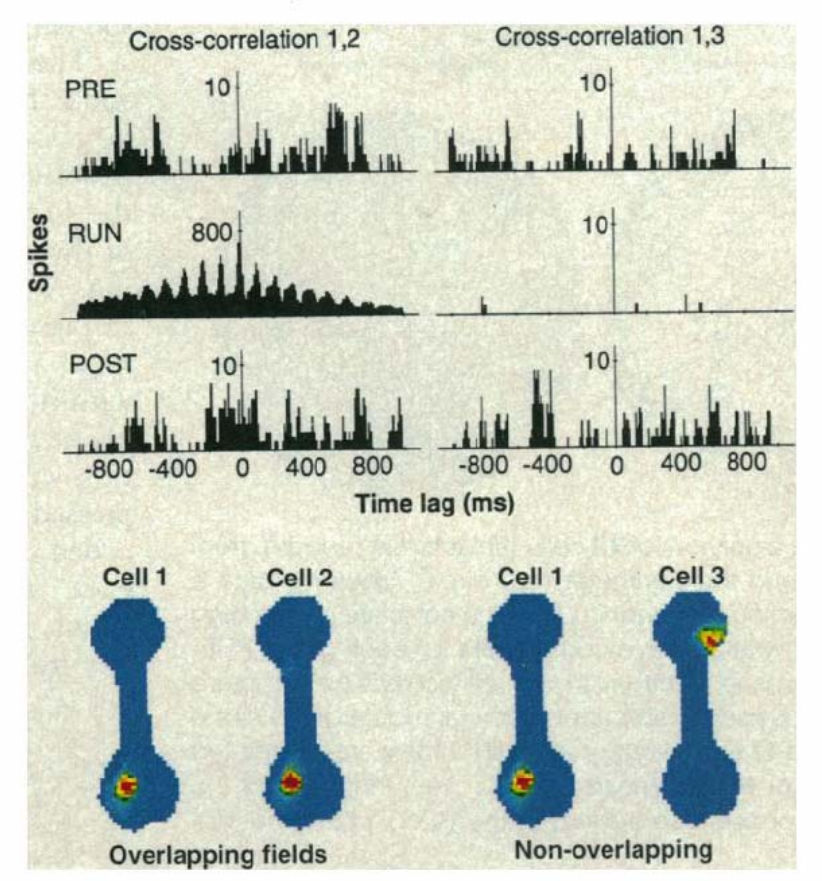

Figure 11: Cross correlations between CA1 cells coding for the same place (left) or different places (right). (From Wilson and McNaughton, 1994).

In a PET study with humans, Peigneux and colleagues (2004) have shown higher activity in the hippocampus and parahippocampal gyrus during slow wave sleep (SWS) in subjects who had done a spatial learning task relative to subjects who had done a simple reaction time task. Besides, retrieval performance increase from day 1 to day 2 correlated with the activation in spots in the bilateral parahippocampal gyrus during SWS, but not during REMS.

Plihal and Born (1999) have shown that low cortisol levels observed in SWS are important for memory retention during sleep: Additional cortisol administered in the first half of the night lead to diminished performance in pair associate learning, but not in a procedural task (mirror tracing).
Gais and Born (2004) have shown a profound influence of low acetylcholine (ACh) levels on declarative memory consolidation during sleep: The whole beneficial effect of sleep on a word pair associate task could be abolished by administration of physostigmine, an acetylcholineesterase inhibitor. The agent was without effect on a procedural learning task (mirror tracing). In waking subjects, no effect was observed. The authors conclude that probably a low ACh level during slow wave sleep in change with high ACh levels during REM sleep promote memory consolidation.

Several studies tried to correlate sleep *spindles* with consolidation. Sleep spindles are very regular characteristic sinusoid waveforms occurring at preferentially in sleep stage 2, which are astonishingly synchronous over the whole cortex. They are triggered by an intermediate hyperpolarisation in thalamocortical neurons. Spindles over medial prefrontal areas have been shown to be tightly correlated with the occurring of hippocampal sharp waves (Siapas and Wilson, 1998). Hippocampal spikes precede prefrontal spindles. It is not clear if both are synchronised by ascending brainstem-thalamic pathways, or if hippocampal outputs directly trigger the spindles in PFC. Spindles could eventually lead to improved conditions for LTP. Taken together with the results of Wilson and McNaughton (1994), this points on a correlation of spindles with memory-related activity during slow wave sleep. Sejnowski (2000) hypothesized that spindle activity opens ‘the gates for molecular

synaptic plasticity', probably over Ca^{2+} entrance and CaMKII, while brief periods of fast oscillations could tag synapses carrying new associations by short-term potentiation. An interplay between spindle activity and bursts could explain the selective strengthening of synapses overnight.

Direct evidence for involvement of spindles in consolidation has been found in a study by Gais and colleagues (2002). They compared spindle activity in subjects who had fulfilled an associative word learning task, compared with subjects who had done a letter comparing task in the same word stimuli. Spindle activity was increased in the learning group in sleep stage 2. Spindle power correlated with retrieval success in the learning group. Another study found a significant correlation between spindle density and performance improvement over the sleep period, but no generally increased spindle density in the night after a word pair learning task compared to the baseline task (a odd letter finding task) (Schabus et al., 2004). A third study found correlations of the all-night numbers of sleep spindles with verbal memory performance (predominantly over left central to frontal electrodes) but no correlation with face recognition (Clemens et al., 2005). Interestingly, decrease of spindle density is a feature of Alzheimer's disease (even at a quite early stage) and other dementias (Montplaisir et al., 1995).

Several studies have shown an influence of *REM sleep* on learning (Smith, 1995): The animal literature is quite consistent and shows similar effects with different types of task, with a greater effect in simple tasks ('reference memory') than in more complex tasks ('working memory'). The results of human studies are less consistent, with big differences across tasks: Declarative memory tasks show practically no effects; procedural tasks show quite consistent effects (but they are difficult to interpret, because under 'procedural' there are tasks as diverse as the tower of Hanoi task or word stem completion or Corsi block tapping), while simple motor task could rather be influenced by stage 2 sleep deprivation (motor pursuit; but it seems methodologically difficult since this was concluded from differences in selective REM deprivation and 2^{nd} half of the night sleep deprivation; stage 2 sleep can not be disrupted without disrupting SWS and REM sleep). It seems to be difficult to conduct well-controlled studies with matched S (sleep drive) and C (circadian) processes (Borbély, 1982). Stickgold (2005) stresses an important difference in the result pattern of different motor tasks: In the serial reaction time task, a more explicit

instruction leads to improvement during sleep only, while an implicit instruction leads to improvements also during waking periods. Imaging studies point also on strategy changes during sleep in motor tasks. For declarative memory, a difficult issue is to show that improvements after a sleep period compared to a waking period is not due to less interference from perceptions made while awake (Stickgold, 2005).

It is also not clear which kinds of consolidation are strengthened by sleep: Systems consolidation or cellular consolidation. The 'replay' studies (Wilson & McNaugthon, 1994), speak for systems consolidation. But also cellular consolidation seems to play a role: Several genes important for memory are upregulated during sleeping, like the calmodulin-dependent kinase IV (CaMKIV) and calcineurin, and zif-268, an immediate early gene which participates in activity-dependent synaptic plasticity (reviewed in Stickgold, 2005). Experimental induction of LTP in the hippocampus leads to zif-268 activation in the amygdala, entorhinal cortex and auditory cortices, later also in somatosensory and motor cortices.
The influence of sleep on system consolidation is extremely interesting and difficult to measure, because newly learned materials interact with the structure of the knowledge a subject already has, which is difficult to measure. One study sheds light on these issues: Ellenbogen and colleagues (2006) succeeded in showing that sleep after learning a first list of word pairs protects from associative interference (A-B-> A-C) by learning of a second list with different associates. This shows an effect of sleep on systems consolidation.

1.3 Memory Genes

Genetics has been a booming field since practically the whole human genome has been deciphered. Unfortunately, this was the smaller part of the work. Now we know the sequence of half a dozen persons and the genetic variants that happened to be found between them. Many more variants will be found in the future. The definition of a gene from the sequence is already not straightforward in all cases. But the difficulty starts if we search for the functions of each individual gene. The advantage of the deciphered genome is that it is possible to find a gene if you have the sequence of a candidate protein which is identified to do something (like being involved in an

illness). Genetic research is shifting from diseases to normal variants, which influence cognitive functions. Of course the action of genes on cognitive functions like memory is always indirect: Most of the genes code for transcription factors (chapter 1.1.2) or signal molecules, which regulate the concentration and distribution of other proteins. The influence of genes on memory is likely to be mediated by brain structure and single cell function. In most of the genes expressed in the brain, we do not even know what they are doing exactly on the level of neurophysiology. Until now, this field of research often works unsystematically and just tries to involve always the same genes in different disorders and functions (BDNF is such an example). Until now, candidate genes often are known from one of two fields: Either they have been involved in diseases (like APOE, Cyp46) or they are likely to be involved in neurophysiology, like receptor proteins (e.g. the serotonin 2A receptor gene). Often they are likely to influence not a single psychological function, but several ('generalist genes', see Butcher et al., 2006; Kovas and Plomin, 2006). In the following I will briefly review some genes that have been investigated by our group.

1.3.1 The Presenilin 1 (*PS1*) Gene

The presenilin 1 gene is known from Alzheimer's disease (as the name says; in biochemistry, proteins are often named after the first context they have been found to be involved in). From the (fortunately rare) familial cases, most carry mutations in the PS 1 gene, which have full penetrance, which means that every carrier of the mutation will get the disease, if he reaches the age of onset (which is about 45- 50). Presenilin 1 is a molecule of the γ-secretase complex at the inner side of lipid rafts in the cell membrane, which helps to split APP (amyloid-precursor protein) into amyloid β (Aβ). It does not only split APP, but also other molecules like notch, which is important for development. Some mutation in the PS1 gene lead to increased production of Aβ42, which is a form of Aβ that aggregates very fast, which may finally lead to Alzheimer pathology. In a study with a young (20 years) carrier of the C410Y variant of this gene, we found fMRI overactivation in memory tasks relative to age- and education matched controls. Besides, we found first signs of an impaired episodic memory performance at that age, 25 years before he will get Alzheimer's (Mondadori et al., 2006b). Probably this is no normal variant of memory, but a sign of

brain pathology (like neurofibrillary tangles in the medial temporal lobe), but for us it was a good proof of principle to see that memory problems can be seen in terms of compensatory overactivation in the fMRI signal.

1.3.2 The Apolipoprotein E (*APOE*) Gene

APOE is the most important lipid transporter in the brain. There are two frequent polymorphisms, which form three allelic types. The most common form is ε3 (which has arginine at residue 112 and cysteine at residue 158). ε4 is similar to APOE found in monkeys, so its probably the ancestral allele (arginine at residue 112 is switched into cysteine), while ε2 is the rarest form (cysteine at residue 158 is switched into arginine). ε2 and ε4 are both risk factors for certain diseases: ε2 carriers have a higher risk for hyperlipoproteinemia, while ε4 carriers have a higher risk for Alzheimer's disease and cardiovascular disease, especially homozygous ε4/ ε4 carriers. ε2 even seems to have a protective effect (reviewed in Mahley and Rall, 2000). Carrying ε4 could have also some advantages, since intelligence was modestly higher in a sample of young women (Yu et al., 2000), and young knock-in mice carrying the human APOE ε4 instead of mouse APOE showed a stronger LTP than knock-in mice carrying the human APOE ε3 instead of mouse APOE (Kitamura et al., 2004). Interestingly, ε3 and ε2 could have evolved in parallel with the longer life expectancy in human beings, to support healthy aging. There should be an evolutionary pressure on humans to grow older to help to grow up grandchildren (*grandmother genes*; Finch and Sapolsky, 1999).
Our study indeed showed better memory in ε4 carriers: In a large sample of students and trainees, ε4 carriers showed a slightly better memory performance, and in a subsample of 34 ε2, ε3 and ε4 carriers, ε4 carriers showed less retrieval-related fMRI activation to reach the same memory performance and decreased their activation if they had to learn the same face-word associations three times, while the other genotypic groups increased activation up to run 3. We interpreted in terms of better memory abilities in ε4-carriers (Mondadori et al., 2006).

1.3.3 The Cytochrome P450 46A1 (*Cyp46*) Gene

The cytochrome pigment 450 proteins are a family of liver enzymes ("hydroxylating monooxygensases"). The cyp 46 A1 enzyme is expressed in the brain and can hydroxylize cholesterol to 24S-hydroxycholesterol (24-OHC). This is important for the cholesterol turnover (degradation; cholesterol stays in the brain for several years until it is replaced) in the brain, because cholesterol is not soluble in blood, while 24-OHC is soluble, so it can be transported to the liver and further catabolized (deconstructed) there. Possibly too high cholesterol in the brain is a risk to get Alzheimer's, so it could be that Cyp46 has an influence on getting Alzheimer's. The catabolite 24-OHC has also various functions: It helps to regulate cholesterol anabolism (construction) over 'liver X-receptors'. It was reported that in Alzheimer's patients, the concentration of 24-OHC correlated positively with a good mental status (Lütjohann et al., 2000). There is also another way how Cyp46 could influence memory: Cyp 46 degrades also sterols like estrogen, testosterone or cholesterol, which influence memory performance.

Cyp46 is a complicated gene with 15 exons. An intronic polymorphism, rs754203, has been implicated to be associated with Alzheimer's disease (Papassotiropoulos et al., 2003): Homozygous TT carriers should have a higher risk than CC or CT carriers. We wondered if this polymorphism has an influence in young, healthy persons, matched our sample from the APOE studies in two groups (TT versus CC/CT) and found a similar pattern as in the APOE study: non-risk-allele carriers overactivated in fMRI in memory tasks. Finally we resigned from publishing these results, for two reasons: There were no convincing data that TT carriers have a better memory, and there were no data indicating that this polymorphism really changes anything in the expression or distribution of the gene. Papassotiropoulos (personal communication) finally supposed that this polymorphism is not causing anything, but was in our population in a linkage disequilibrium (correlated) with another polymorphism in the promoter of the gene, which has an effect on the risk of getting Alzheimer's. However, this polymorphism was never found.

1.3.4 The Serotonin 2A Receptor Gene

The serotonin 2A receptor gene is highly interesting for learning (see chapter 2.1.3/ 2.1.4). Serotonin is an indoleamine implicated not only in pain, but also in mood and depression. It is well-known that memory is impaired during depressive episodes. One study directly showed effects of tryptophan depletion on subjects undergoing a memory task in fMRI (van der Veen et al., 2006). Tryptophan is the precursor for serotonin, so tryptophan depletion leads to decreased serotonin in the brain. During encoding, dietary tryptophan depletion reduced right hippocampal activation, and retrieval performance was impaired as compared to the same subjects on another day (crossover design). In wide parts of the brain, tryptophan depletion led to compensatory overactivation.

There is a His452Tyr polymorphism in the serotonin 2A receptor gene. The serotonin 2A receptor is expressed strongly in the cortex, especially the frontal cortex (Buhot, 1997). Platelet cells of His/Tyr carriers show a blunted receptor response upon pharmacologic stimulation with serotonin (Ozaki et al., 1997; Göthert et al., 1998). Our group has shown strongly (21%) reduced word learning in 452 His/Tyr carriers after delays of 5 minutes and 24 hours (the pattern does not look like a consolidation effect, but like an effect of early episodic memory, because the difference does not get larger between 5 minutes and 24 hours after learning (Figure 12).

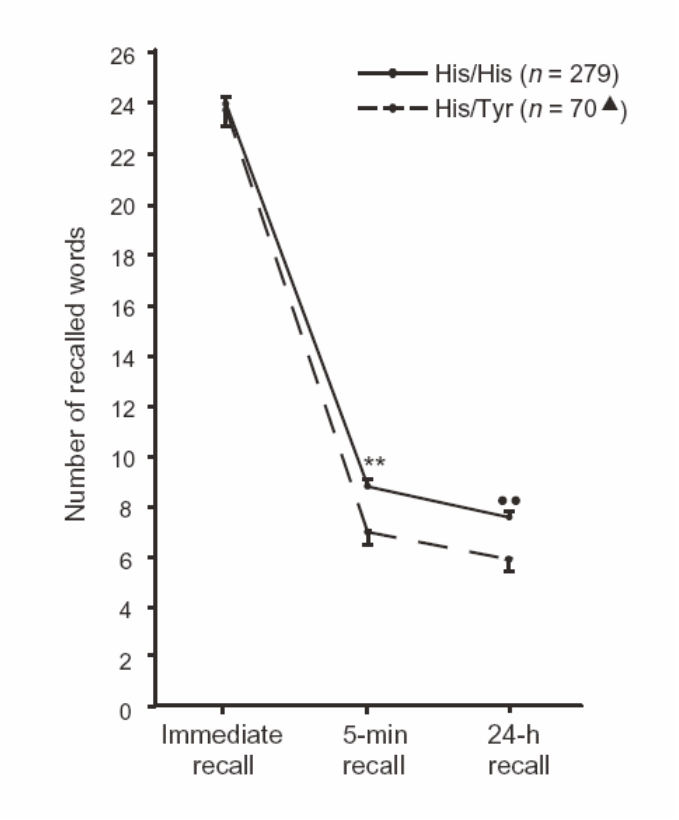

Figure 12: Effect of the His452Tyr polymorphism (de Quervain et al., 2003)

In a later study, Reynolds et al., (2006) showed an effect of a nearby polymorphism (1438 G/A) on deterioration at the age of 65 years in figural memory in a recognition task, but not in two other episodic memory tasks (recall of faces / names).
A volumetric study using voxel-based morphometry and mask-based volume-of-interest (VOI) meaurements found reduced white matter volumes in the temporal lobe and reduced grey matters volumes in the left hippocampus, the left inferior temporal gyrus, and bilaterally in the middle and superior temporal gyrus (Filippini et al., 2006).

1.3.5 The Brain Derived Neurotrophic Factor (*BDNF*) Gene

BDNF was the second neurotrophin which was discovered, and its amino acid sequence is quite similar to nerve growth factor (NGF), the first discovered neurotrophin. Neurotrophins are molecules that can be taken up by neurons and give a “survival signal” to the neuron. This process is important for neuronal plasticity, because during development, too many neurons are grown, and the brain has to choose which ones have to die (*apoptosis*). BDNF is expressed strongly in the brain, first of all in hippocampus, amygdalae and cortex (see Murer et al., 2001 for a review). BDNF acts on all sorts of neurons and was implicated as a therapy against Alzheimer’s and Parkinson’s disease, because BDNF expression is downregulated in these diseases. The importance of this protein can also be seen in its fine-tuned genetic regulation: There are 8 different versions of mRNA, with 4 different promoters and 2 different kinds of untranslated region at the end (Timmusk et al., 1993), though the protein stays always the same.
BDNF is probably involved in memory function: Knockout mice have impaired long term potentiation (LTP), and LTP can be rescued by reexpression of BDNF in neuronal cultures (Korte et al., 1996).
There is a common polymorphism (Val66Met) in the *pro-region* of the protein. The pro-region is a part of the protein that is always translated from the mRNA, but cleaved off at the destination of the protein. The pro-region is used by the protein to find its right place, like an address on a postcard. So the polymorphism does not

change the protein itself, but its distribution (Egan et al., 2003): valBDNF is expressed in the soma and dentrites of neurons, whereas metBDNF stays mainly in the soma. This has probably consequences for the survival of presynaptic cell, since BDNF works as a *retrograde messenger* that is taken up by the axon and transported back to the nucleus, where it acts as a transcription factor. Consistent with the above findings, humans with a Met-allele had showed a lower metabolism in the left hippocampus (as measured by NAA= N-acetyl-aspartate; a marker for neuronal function which can be used in MR-spectroscopy) and a lesser memory performance in a *dose-dependent manner* (which means, Met/Met<Val/Met<Val/Val; Egan et al., 2003). The same group found a lower activation of Val/Met carriers than Val/Val carriers in fMRI during a visual episodic memory task (Hariri et al., 2003).
We investigated the same polymorphism in the sample from the APOE study (Mondadori et al., 2006), with inconsistent results. However we compared grey matter with a manual volume-of-interest account and with voxel-based morphometry and found a tendency of reduced grey matter volumes in the medial temporal lobe and significantly reduced grey matter volumes in wide parts of the brain in Val/Met carriers relative to Val/Val carriers (Figure 13).

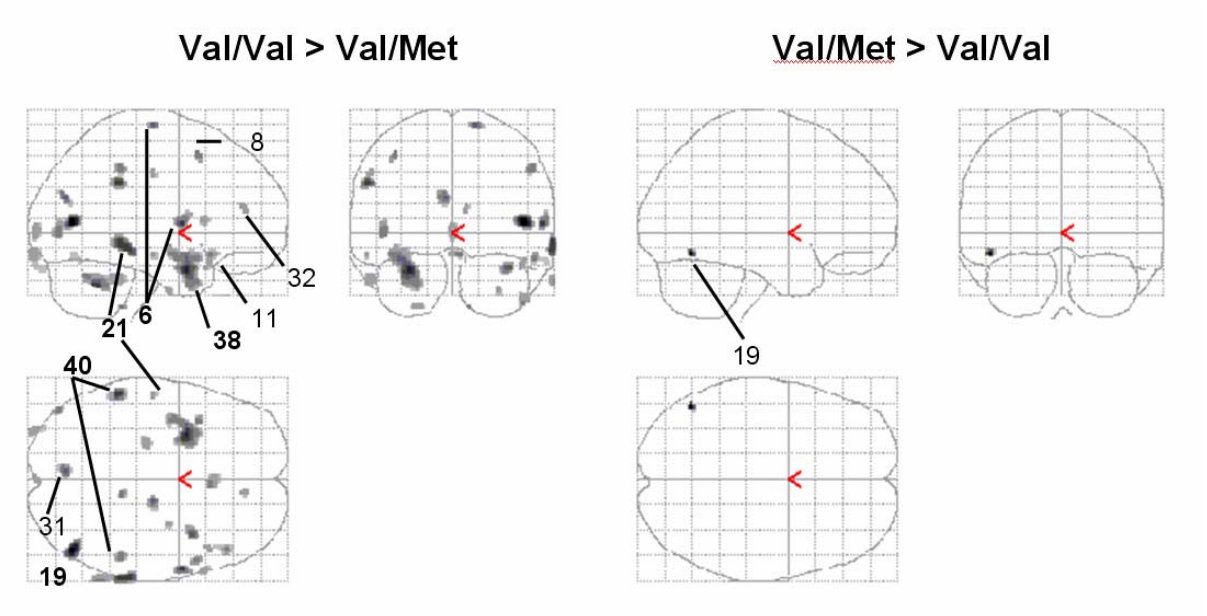

Figure 13: Glass brains showing voxels (volume elements) in the brain where Val/Val carriers have more grey matter than Val/Met carriers (left) and vice versa (right)

Two weeks after doing these calculations, a study was published (Pezawas et al., 2004) with approximately the same results (also smaller volumes in Met-carriers, but the areae of the significant spots only partly overlapped: we did not find occipital spots, but more temporal spots).

1.3.6 The *KIBRA* Gene

A genome-wide search among 502627 single nucleotide polymorphisms with pooled blood of subjects of 4 performance classes yielded two polymorphisms that were significant even if corrected for multiple comparisons. As the two polymorphisms were tested with a second population, only the polymorphism rs17070145 in the ninth intron of the KIBRA gene was still significant (Papassotiropoulos et al., 2006): T allele carriers had significantly better memories for words than homozygous CC cariers, despite of statistically equal working memory (mean CC carriers: 7.6 (SEM 0.2); mean CT/TT carriers: 9.4 (0.2)). The KIBRA protein has been found to be a binding partner of dendrin, a putative modulator of synaptic plasticity (Kremerskothen et al., 2003). A truncated form expressed in the hippocampus contains a domain interacting with protein kinase C zeta (PKCζ; Buther et al., 2004) which has been implicated in LTP maintenance (Sacktor et al., 1993, Drier et al., 2002).
In our face-profession association fMRI task (Mondadori et al., 2006) with performance-matched KIBRA genotypic groups, CC carriers showed more activation than CT/TT carriers in wide parts of the brain, in the hippocampus (Figure 14), frontal cortex (Brodmann's areae 6, 8 and 9) and in the parietal cortex (BA 40). This higher activation to reach the same behavioral level (in our interpretation) is consistent with the worse memory of the CC carriers.

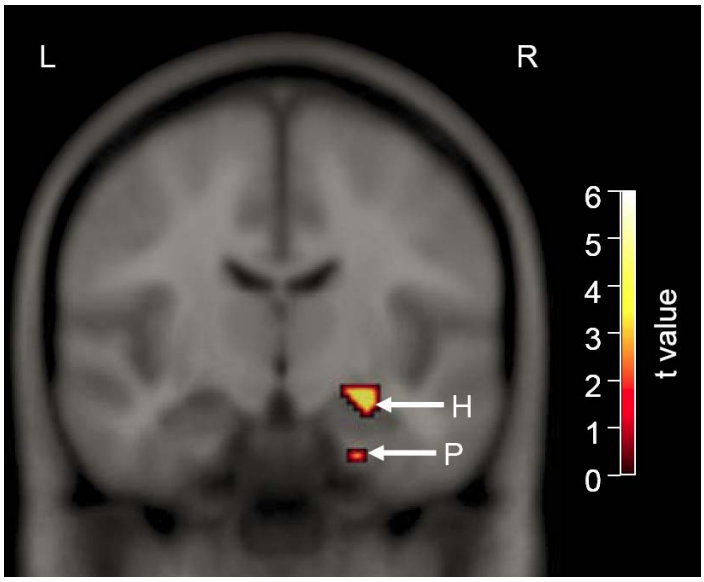

Figure 14: Overactivation of CT/TT carriers over CC carriers in the medial temporal lobe (Papassotiropoulos et al., 2006)

1.3.7 The Prion Protein Gene (*PRNP*)

This gene will be introduced a second time in the introduction of the paper in chapter 2. Here we will summarize some aspects that were not touched or only very short in the paper. The term *prion* (derived from “protein” and “infective”; Prusiner, 1982) first circumscribed with “proteinaceous infectious particles that resist inactivation by procedures that modify nucleic acids” (Prusiner, 1998) or “a proteinaceous infectious particle that lacks nucleic acid” (Prusiner & Scott, 1997). From a broader perspective, a definition could also be “elements that impart and propagate conformational

variability" (Prusiner, 1998). There are three hypotheses about how the infectious agent could look like (Oesch et al., 1985): The most important one is the "*protein only hypothesis*". It states that a prion is a special form of a normal protein, which is able to impose its conformational state to the normal form of the protein. The infectious form is called PrP^{SC}, (SC for "scrapie"), the normal cellular form PrP^{C}. There are also two other hypotheses, the "virino hypothesis", which assumes that the infectious agent consists of a scrapie-specific genome coated with host-derived PrP^{SC}, and the "conventional virus" hypothesis, which assumes that the infectious agent is a virus with unusual properties (Weissmann, 1996; 2004). The virus hypotheses are constrained by the fact that there is an upper bound in molecular weight for the agent.

In a seminal paper, it was shown that the sequence of the infectious protein agent is coded by a cellular gene (Oesch et al., 1985). Then it was found that this amino acid sequence corresponds with the mRNA of a mouse or hamster protein found in uninfected animals, ("PrP^{C}") (Chesebro et al., 1985). Later it was confirmed that they are encoded by the same gene, and concluded that their different properties must be due to posttranslational events (Basler et al., 1986). Weissmann (2004) points out that although the resistance of PrP^{C} knockout mice (see below) confirms the 'protein only hypothesis', it has never been proved directly (i.e., by showing that pure prions can infect an animal).

1.3.7.1 Prions in Fungi

At least 5 molecules have been found to have prion-like properties (namely conformational switch into a more protease-resistant form): The prion protein PrP and three fungal 'prions', namely the Sup35 protein (leading to the psi^{-} - PSI^{+}-switch, see below) and the Ure2p protein (leading to the URE3-phenotype, causing to a deregulation of nitrogen catabolite repression) in yeast, and the Het-s protein in Podospora, the switch of which seems to be functional to limit virus spread (reviewed

in Brockes, 1999). Another functional prion in yeast seems to be the β-protein (see below).

There are 4 principially different ways in which prions act (Figure 15): Some trigger a toxic cascade (PrP^{SC}), some are connected with loss of function (URE3, PSI), some with a gain of function (Het-s), and some have a self-activating action (β-enzyme).

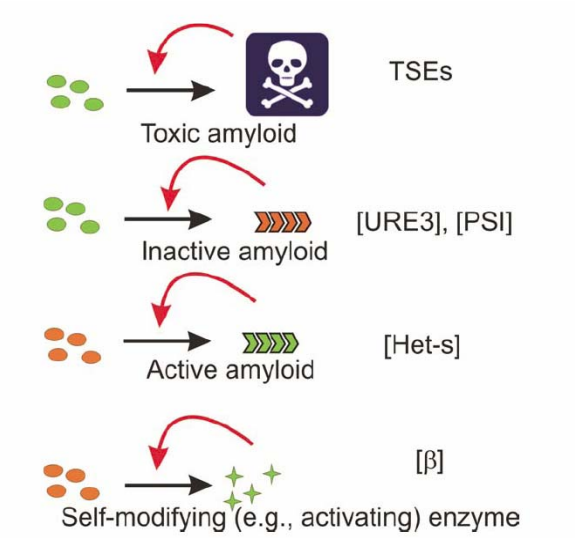

Figure 15: Ways of action of different prions (from Wickner et al., 2004)

The PSI^+ phenomenon in yeast switches cells into an altered state by a prion-like mechanism: *Sup35* is a protein that suppresses transcription together with Sup45. In a psi^- cell, Sup35 is soluble and able to suppress translation of nonsense mutations. In PSI^+ cells, most of the Sup35 is insoluble, and its insolubility is inherited from generation to generation. The lack of soluble Sup35 leads to occasional translation of nonsense mutations.
A similar *cytoplasmatically inherited* genetic element (which means, the information is not in the DNA, but in the cell), [URE3], exists in yeast (Wickner, 1994). Yeast (saccharomyces cerevisiae) cells use the Ure2/URE3-protein to regulate nitrogen metabolism: Under nitrogen-rich conditions, Ure2 binds to a transcription factor and prevents its entry into the nucleus. Under nitrogen-poor conditions, Ure2 is switched into URE3-amyloid aggregates, so there is not enough Ure2, and the transcription factor comes to action and triggers changes in nitrogen metabolism.

HET-s is a prion of the fungus podospora. The HET-s protein regulates compatibility between genetically identical or different cell colonies. There are two alleles in the het-s locus, het-s and het-S. The het-s allele exists in two forms: The prion protein HET-s, which is incompatible with HET-S, and the non-prion protein HET-s*, which is compatible with HET-S. Hence, the prion form is responsible for the lethal incompatibility reaction. HET-s is self-propagating and digested less by proteinase K than HET-s* (Coustou et al., 1997). HET-s and HET-S differ in 13 amino acids, 2 of which are most critical. Proline at codon 33 instead of histidine and asparagin acid instead of alanin at codon 23 enables prion switching. In contrast to the other mentioned prions, the prion-domain of HET-s is C-terminal and not Q/N-rich (reviewed in Wickner et al., 2004).
β is another prion in yeast. It is a self-activating form of the vacuolar protease B (coded by the PRB1-gene) and can be transmitted from one cell of a syncytium to the other. The β-stage is crucially dependent on the concentration of the protein. Cells without β are not able to undergo meiosis and to produce spores and are more prone to starving, so β has definitely an advantageous effect (Wickner et al., 2004).
In their review-paper, Wickner and colleagues (2004) point out that fungi and plants are likely to have many different prions, because they would enable rapid switches upon environmental changes over their syncitial/interconnecting structures.

CPEB and long term memory:

CPEB (cytoplasmic polyadenylation element binding protein) regulates mRNA translation. It acts by elongating the poly-A-tails of dormant mRNA. Most of the time, CPEB activates mRNA, but sometimes it represses mRNA. The activation of CPEB is normally controlled by phosphorylation, but the neuronal isoform of Aplysia CPEB lacks the normal phosphorylation sites. Si and colleagues (2003a) have found that a neuronal isoform of the Aplysia CPEB has prion-like properties: It has a region very rich in glutamine (Q) and asparagine (N), shows high conformational flexibility (not one stable secondary structure) and can be found as soluble species or self-perpetuating aggregates. In vitro, only the aggregates were able to bind CPE, the soluble proteins were not.
These issues were taken up by another study by Si and colleagues (2003b): It was shown that pulses of serotonin (5HT) in aplysia induce long term facilitation (LTF)

and CPEB-expression in parallel. Interestingly, injection of CPEB-antisense oligomers abolished the long term (72h) effect, but not the short term effect (24h) of 5HT. So probably the protein is not necessary for marking a synapse (PKA-dependent process), but for maintenance of the marking (rapamycin-dependent process). The same phosphorylation-independent “neuronal” form of CPEB has been found in mice, men and flies.
Shorter and Lindquist (2005) point out that such a mechanism could explain how molecular memory traces persist on much longer time-scales than the molecules involved. This mechanism probably works better than other mechanisms proposed for the molecular correlate of memory traces: autophosphorylation loops, self-sustaining activity loops in complex signalling networks or transcription factors that stimulate their own synthesis.

1.3.7.2 Biological Chemistry of the Prion Protein

The prion protein (PrP) is a sialoglycoprotein found predominantly on the surface of neurons, attached by a glycosylphosphatidylinositol (GPI) anchor.
The *primary structure* (Figure 16; the sequence of aminoacids) of the prion protein is quite similar in humans, rats, mice and hamsters; sheep and bovine protein is somewhat more different; most different is the chicken protein (Prusiner & Scott, 1997). The protein consists of an N-terminal region with 5 octarepeats, a long hydrophobic and very homologous region in the middle and the GPI-anchor at the C-terminal side (Stahl et al., 1987), which can be released by PIPLC (phosphatidylinositol-specific phospholipase C) in PrP^{C}, but not in PrP^{SC} (Stahl et al., 1990; Whittington et al., 1995). The N-terminal side contains a signal sequence which targets the protein to the endoplasmatic reticulum (ER), where the peptide is removed (Stahl & Prusiner, 1991).
Biosynthesis of PrP is completed in several steps: The 22 N-terminal and the 23 C-terminal aminoacids are cleaved, two Asn residues are glycosylated, and a glycosyl phosphatidyl-inositol (GPI) anchor is attached to Ser231 (Weissmann, 1996).

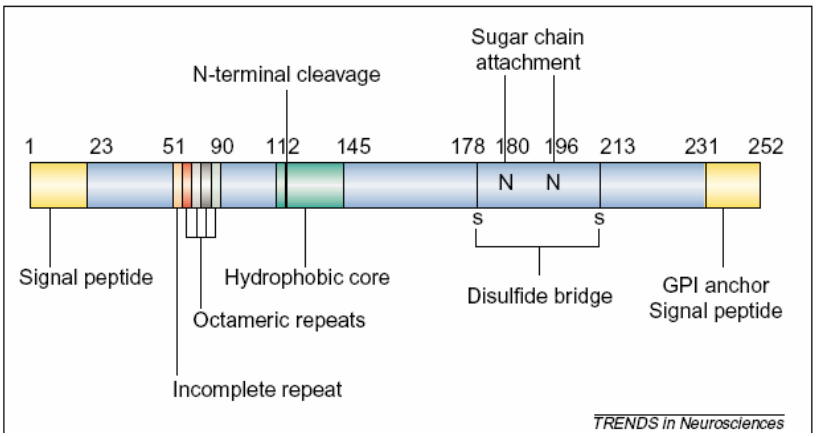

Figure 16: PrP primary structure (from Brown, 2001)

Although PrP^{C} and PrP^{SC} probably have identical primary structures, their secondary structures are markedly different (reviewed in Prusiner, 1998): PrP^{C} contains about 40% α-helix and little β-sheet, while PrP^{SC} contains about 30% α-helix and 45% β-sheet. Figure 17 shows plausible secondary structures of PrP^{C} and PrP^{SC}.
Copper binding imposes a more regular (α-helical) strucure to the octapeptide repeat part in the N-terminal tail of the protein (Miura et al., 1996). High copper-concentrations support endocytosis of PrP from the cell surface (reviewed in Unterberger et al., 2005).

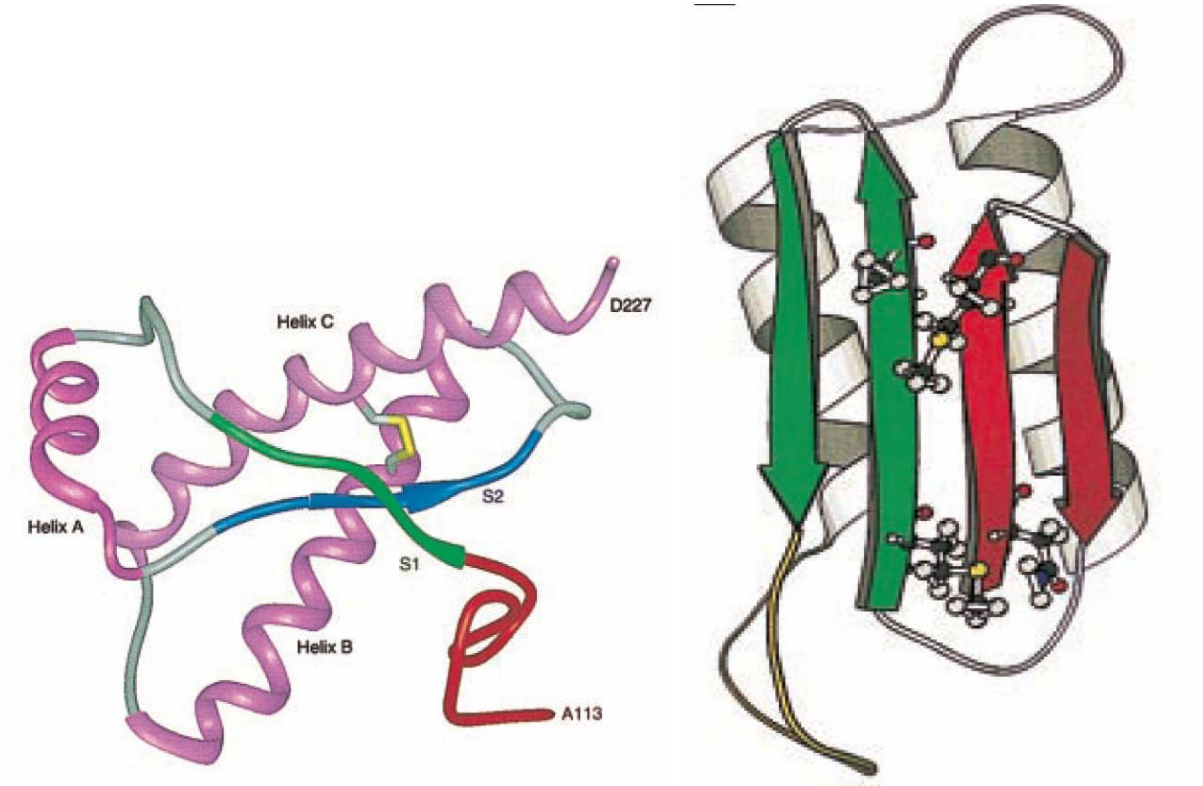

Figure 17: Plausible secondary structures of PrP^{C} (left) and PrP^{SC} (right) (from Prusiner, 1998). Corkscrew signatures are α-helices, arrows are β-sheets.

There are at least 5 variants of the protein (Brockes, 1999; Ermonval et al., 2003; Harris, 2003): The normal form is GPI anchored (normal and N-terminally truncated form). The *secretory* form (Hay et al., 1987), ^{SEC}PrP is the precursor of the GPI-anchored molecule, but can sometimes be encountered in the cell lumen. The *transmembrane* form has two conformations, one with the C-terminal end towards the cell lumen ('^{ctm}PrP'), and one with the N-terminal end towards the cell lumen ('^{ntm}PrP'). Both forms span the cell membrane with the same stretch of amino acids, *TM*1 ('TM' for transmembrane; AA111-134). ^{ctm}PrP is enriched in certain familial prion diseases (in certain mutations in the transmembrane region) and has been claimed to be toxic (see below or Hegde et al., 1998, 1999).

Expression of the cellular form (PrP^{C}) has been shown in wide parts of the brain, predominantly in neurons, following a size effect: Large neurons have more PrP^{C} than small ones (with the exception of some Purkinje cells in the cerebellum). The

distribution over brain structures seems to be rather uniform and does not significantly change following scrapie infection (Kretzschmar et al., 1986). Expression in the brain is strongest in the hippocampus (about 10x more than in spinal cord or cerebral cortex), somewhat weaker in the septal nuclei and in the nucleus caudatus, and even less in thalamus and in subthalamic nuclei and in the spinal cord. Olfactory bulb and cerebellum showed very little staining (Bendheim et al., 1992). Staining is localised in some cells and especially in the neuropil (compared to the diffuse staining of PrP^{SC} in the extracellular space; Bendheim et al., 1992). Another group found PrP^{C} immunoreactivity in the hippocampus, the entorhinal cortex, the ventral striatum and the substantia nigra (Moya et al., 2000; see also Figure 18). The authors conclude that PrP^{C} is found in synapse-rich areas of highly plastic structures. The staining was found pre- and postsynaptically (Moya et al., 2000), while immuno-electron microscopy studies showed it to be predominantly in presynaptic membranes (reviewed in Fournier et al., 2000). Another group observed more PrP^{C} in the deep layers of the cortex compared to the superficial layers; is also expressed in white matter and glia, with a rostrocaudal decrease (Moleres et al., 2005).

There are two different ideas about subcellular PrP^{C}-distribution (Schmitt-Ulms et al., 2001): One assumes concentration in calveolae-like domains in lipid rafts (see e.g. Vey et al., 1996), the other a more wide and homogeneous distribution in the membrane. PrP^{C} seems to be taken to the synapse over anterograde transport in a special glycosylated form (Rodolfo et al., 1999). At least sometimes PrP^{C} can also be found in the nucleus (reviewed in Ermonval et al., 2003). Mironov and colleagues (2003) found most PrP is in the (presynaptic and postsynaptic) plasma membrane and on the way there (ER, endosomes), but none in synaptic vesicles, the mitochondria or in clathrin-coated pits. They were enriched in synapses, but seemed to move over the whole membrane and not to be bound to certain compartments. Interestingly, some 1-2% of the neurons showed PrP in the cytosol. These cells looked normal (not necrotic or apoptotic) and were found in the hippocampus, thalamus and somatosensory cortex. The authors conclude that they probably are a second isoform than the one found in plasma membranes.

In the peripheral organs, PrP^{C} is expressed primarily in lungs, heart, spleen and skeletal muscles, but hardly in the liver (Bendheim et al., 1992).

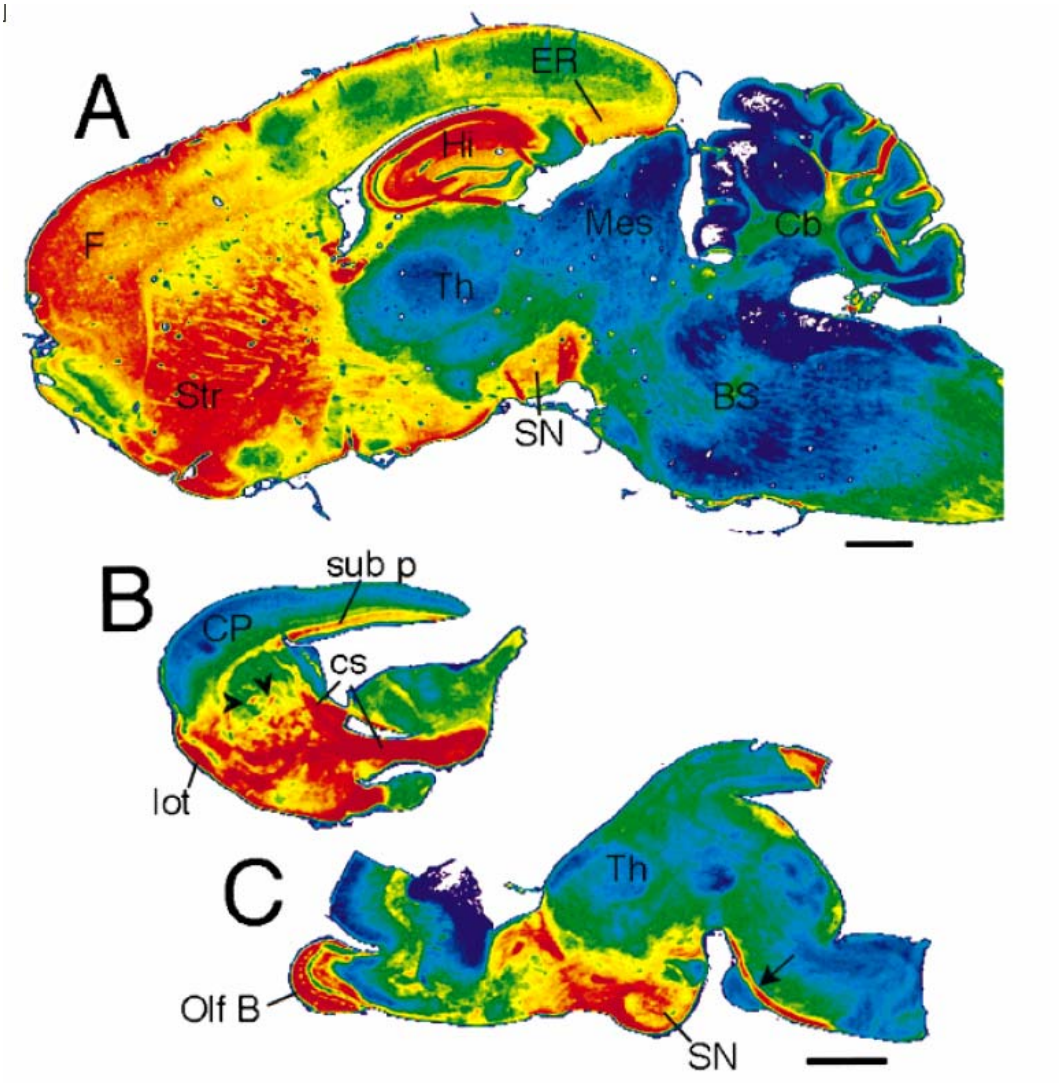

Figure 18: Expression of PrP in the hamster brain (A adult, B&C postnatal day1) (from Moya et al., 2000); F: frontal cortex; ER: endoplasmatic reticulum; Hi: hippocamus; Th: thalamus; Mes: mesencephalon, Cb: cerebellum; Str: striatum; SN: substantia nigra; BS: brain stem; subP: subplate; CP: cortical plate; sc: corticospinal tract; lot: lateral olfactory tract; Olf B: olfactory bulb

In a developmental study, Salès and colleagues (2002) systematically looked at PrP^C expression (stained with the antibody mab3F4) in the maturing hamster brain. In most regions, expression peaked around postnatal day P21 (Figure 19). In general, only neurons were stained. Staining started shortly after neuronal differentiation (e.g. embryonic day 14.5 for retina, olfactory bulb and cortical plate). First, there was a staining of axons (during their elongation), then maximal staining shifted to synapses

and neuropil (in adults). Regions developing early, e.g. the olfactory bulb and the striatum, showed the adult staining pattern at birth already.
Mobley and colleagues (1988) showed that injection of NGF (nerve growth factor) in neonatal hamster medial septum raised PrP^C-levels in cholinergic neurons dramatically (x10) and ChAT (choline acetyl transferase; an enzyme connected with action of the neurotransmitter acetyl choline) in parallel (x2).
PrP^C staining seems to be strongest in plastic regions, which can also be seen in Figure 19, since the olfactory bulb and hippocampus, regions where cell proliferation still takes place in the adult, remain highly stained, while all the other regions already diminish their PrP^C activity.

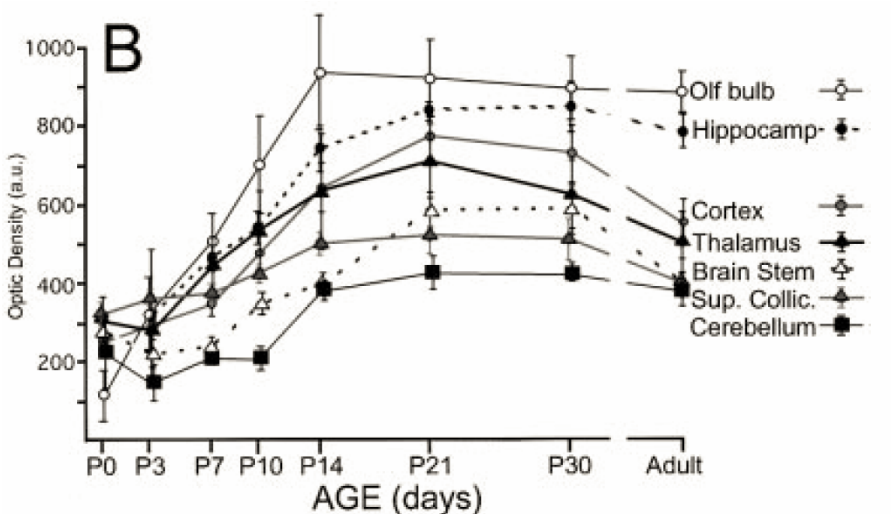

Figure 19: Time course of PrP^C expression in the hamster brain (from Salès et al., 2002)

1.3.7.3 Features of the Prion Protein Gene (*PRNP*):

The human prion protein gene (*PRNP*) can be found in a single exon on the short arm of chromosome 20 (Stahl & Prusiner, 1991). There are one or two additional, noncoding exons upstream of this big ‘exon 3’; most mammals have 3 exons, while in humans, the second exon is hardly ever expressed, so the mRNA consists only of

the sequences encoded by exons 1 and 3. Figure 20 shows the intronic and exonic polymorphisms around the prion gene (*PRNP*), including the doppel gene (*PRND*).

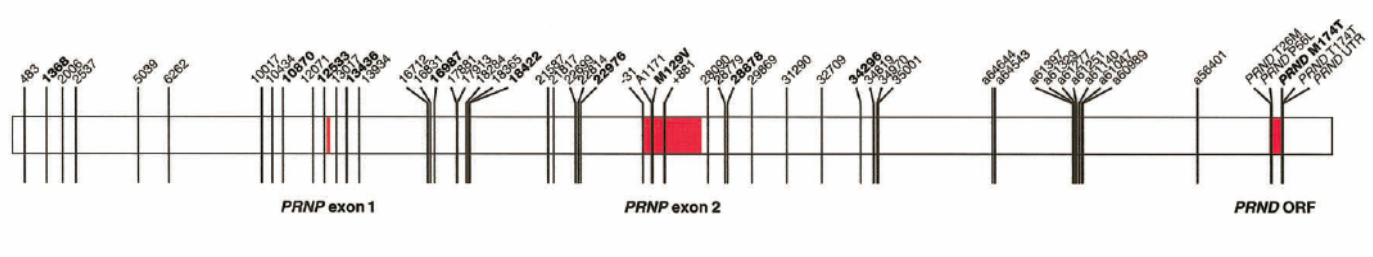

Figure 20: Location of SNP's in the vicinity of the prion gene (from Mead et al., 2001)

The doppel protein is similar to the prion protein, but has no octarepeat region (which is important for copper binding). Doppel is expressed very weakly in the adult mouse brain, but strongly in the testis (male mice lacking doppel are infertile; Behrens et al., 2002). During development, there are higher levels in the CNS (Moore et al., 1999; Silverman et al., 2000).

Prion protein knockout (*PRNP*$^{0/0}$) mice:

(A 'knockout mouse' is a mouse lacking a certain gene that has been destroyed before development). *PRNP*$^{0/0}$ mice have been shown by different groups to be highly resistant against scrapie-infection (Büeler et al., 1993, Prusiner et al., 1993). The susceptibility can be reintroduced with transgenes: Syrian hamster transgenes in *PRNP*$^{0/0}$ mice made them susceptible to hamster prions.

These *PRNP*$^{0/0}$ mice look quite healthy, despite no measurable protein or mRNA concentration (Büeler et al., 1992; Manson et al., 1994).

1.3.7.4 Prion Diseases

They are biologically unique in that they can be simultaneously infectious and inherited. These diseases include Creutzfeldt-Jakob Disease (CJD), Gerstmann-Sträussler-Scheinker Syndrome (GSS) and Kuru in humans, Scrapie, BSE (Bovine Spongiform Encephalopathy) and Chronic Wasting Disease (CWD) in animals. After

a long latent period, “transmissible spongiform encephalopathies” (TSE) are rapidly processing, fatal neurodegenerative diseases (Glover et al., 1997).
TSEs are spread by prions, which appear to consist only of a plasma-membrane protein (“protein only hypothesis”) with a normal conformation, PrP^{c}, and a pathological conformation, PrP^{sc}. The pathological conformation seems to be dominant and induces a conformational switch in PrP^{c}->PrP^{sc}. Cardinal difference of PrP^{sc} from PrP^{c} is its resistance to proteinase digestion and its insolubility. Upon treatment with proteinase K, PrP^{c} is degraded completely, whereas PrP^{sc} loses an N-terminal domain with the octarepeats and yields PrP 27-30, which forms rod-like structures which behave as amyloid (unbranched fibrils, stained with Congo red, cross β-pleated sheats). Otherwise, no chemical difference between the two proteins has been reported (Stahl & Prusiner, 1991).
The mechanism of *prion replication* is not fully understood. There are two models, the “*refolding*” model, in which cellular PrP is folded upon the template of a infectious prion (‘heterodimer’ model: Prusiner, 1991) or an aggregate of two or several prions (Griffith, 1967), while in the “*seeding*” model, the infectious prion can be stabilised only in a crystal-like structure, the ‘seed’ (Come et al., 1993).
Neuropathology is regionally highly correlated with the premorbid amount of PrP^{C}. Plaques appear relatively late in the course of the disease (DeArmond et al., 1987). Although infectious agents of different prion diseases can affect different species, prion diseases show a relative ‘species barrier’ in terms of prolonged incubation times if moving from one species to another (Stahl & Prusiner, 1991).
The *mechanism of neurodegeneration* is not understood yet (Aguzzi & Miele, 2004). The debate if PrP^{SC} is toxic at all lead to the idea that there is another toxic form called PrP*. Besides, it could be that the toxicity depends on the aggregation state (Gavin et al., 2005). Brown and colleagues (1996) found out that presence of microglia is needed for neurotoxicity of PrP(106-126), an artificial peptide widely used as a model for TSE pathology. TSE’s lead to more oxidative stress and a weaker response to oxidative stress (reviewed in Unterberger et al., 2005): Accumulation of PrP^{SC} promotes generation of ROS (reactive oxygen species), which upregulates PrP^{C}, which in turn leads to more conversion into PrP^{SC} (vicious circle). Besides, ROS prevent copper binding to PrP^{C} and lead to higher Cu toxicity. In another paper, Brown (2000) found a second pathologic mechanism in prion protein mutated at codon 117 (A117V), a mutation found in GSS (Gerstmann-Sträussler-Scheinker

Syndrome): This mutation destabilises microtubuli, which leads to toxic Ca-influx into the cell over L-type calcium channels. The deposition of neurofibrillary tangles in some case of this and other mutations are interpreted as accumulated tau-protein, which was not able to bind the microtubuli because of the PrP mutation.
According to an alternative view, cell death is more related to PrP^{C} isoforms than to PrP^{SC}: Hegde and colleagues (1998) found in mice with different *PRNP* mutations, that one of two transmembrane isoforms (see above), ^{ctm}PrP (the form in which the C-terminal end is in the ER lumen) can confer severe neurodegeneration. In a patient died of GSS, they found raised ^{ctm}PrP levels but practically no PrP^{SC}. Vincent and colleagues (2001) point out the similarities between the metabolic mechanisms in APP (amyloid precursor protein) and PrP: Both molecules have two kinds of cleavage, an “α”-cleavage leading to neurotrophic products, and a “β”-cleavage leading to potentially neurotoxic products [and amyloids]. In both molecules, the α-cleavage exactly splits up the amyloidogenic part and is done by the same enzymes (ADAM10 for constitutional and TACE for PKC-regulated α-cleavage).

Human Prion Diseases:

Incidence is less than 2 per million and year (Unterberger et al., 2005). Approximately 15% of the cases of human prion diseases are inherited autosomal dominantly, whereas most of the cases (about 80%) are sporadic. A small number of cases come from accidental inoculation (e.g., iatrogenic: for example through corneal transplants or bovine growth hormones; Whittington et al., 1995). A new variant of CJD, “vCJD”, is probably caused by infection with bovine prions.
In sporadic CJD, homozygous 129Met/Met and 129Val/Val (see below) genotypes are heavily overrepresented (Palmer et al., 1991); practically all victims of vCJD were found to be 129Met/Met homozygotes (Andrews et al., 2003).
The “diagnostic triad” for CJD consists of (1) dementia, (2) myoclonus and (3) abnorm periodic EEG (repeated phases of 1s bi- or triphasic complexes: PSWC = periodic sharp wave complexes). Cases with absence of EEG abnormalities AND involuntary movements are practically never CJD. Brown and colleagues (Brown et al., 1986) evaluated clinical courses and symptoms in a large sample of 230 pathologically verified patients in France. 4-8% of the cases were familial. Age of onset varied between 19 and 83 years, with mean 61.5 years. The mean duration of

the illness was 7.6 months, median duration of the illness 4 months. In about one third of the patients, there was a prodromal phase with aesthenia and disturbances of sleeping and eating patterns. Disease onset was fast or even sudden in 20% of the patients. Disease started with dementia in 2/3 of the patients. In 1/3, there were motor complaints before onset of dementia. Further symptoms were mainly cerebellar: gait disturbance, clumsiness, diplopia, bizarre color perception, generalized or hemianoptic loss of vision. In this French population, only 10% of the patients survived more than 1 year. However, there were also long-term cases of up to 10 years. This was very different in a Japanese population, where the disease lasts about twice as long. Rapid onsets correlated with predominantly neurological symptoms, and early onset correlated with long disease courses.
Two independent polymorhisms have been shown to be associated with sporadic CJD: The well-known M129V polymorphism in exon 2, and SNP1368 upstream of exon 1 (Mead et al., 2001; see Fig. 4).

1.3.7.5 Suggested Functions of the Prion Protein in Healthy

We review only functions within the central nervous system. Possibly there are also functions in hematopoietic stem cells (Zhang et al., 2006), and in the peripheral immune system (Cashman et al., 1990; Diomede et al., 1996).

1.3.7.5.1 Astrocytic Glutamate Uptake

Brown and Mohn (1999) have shown that astrocytes lacking PrP^C are slower in glutamate uptake than wild type cells grown under the same conditions. This is important, since glutamate is toxic for neurons. PrP^C-lacking cells were also more sensitive to H_2O_2-induced oxidative stress and additionally reduced glutamate uptake. Cu^{2+}-ions also reduced glutamate-uptake in PrP-lacking, but not in wild-type cells at the chosen concentration. This effect probably acts over oxidative stress by enzymatic products of Cu^{2+}. The authors suppose that low Cu^{2+}-levels in PrP-deficient cells could lead to an ‘internal’ oxidative stress, because protecting enzymes like superoxide-dismutase (SOD) need PrP^C to work properly. Many conditions like

ROS, mercuric chloride, simulated ischemia or hypoxia reduce glutamate uptake by astrocytes, which has the potential to lead to neuronal death. Other authors state that glia do not express PrP^C at all (e.g., Steele et al., 2006).

1.3.7.5.2 Neuroprotection

Kuwahara and colleagues (1999) have shown that expression of cellular prion protein can rescue mouse hippocampal cells from apoptosis in vitro cells, from which serum was removed (compared to comparable cells not expressing PrP^C). Besides, $PRNP^{0/0}$ cells extended shorter neurites than $PRNP^{+/+}$ cells.
Bounhar and colleagues (2001) replicated and extended this finding. They showed that PrP^C could prevent Bax-induced apoptosis in human primary neurons in vitro, while a PrP with excised four octapeptid repeats, or mutated PrP from families with fCJD (T183A, D178N) failed to stop apoptosis. However, cellular PrP^C without the GPI-anchor signal peptide succeeded in preventing apoptosis. Frigg and colleagues (2006) have demonstrated a protective effect against bright light in mouse retinal cells. They compared apoptosis through bright light in PrP knockout, wild type, and two sorts of PrP overexpressing mice. Whereas the overexpressing mice showed practically no retinal apoptosis in a certain luminance condition, wild type mice showed little effect, while in knockout mice there was a high amount of apoptotically died retinal cells. The authors found out that PrP ultimately influences caspase-1, a pro-apoptotic signal molecule.

1.3.7.5.3 Metal and Reactive Oxygen Species (ROS) Metabolism

PrP^C can bind copper or zinc by virtue of its octapeptide repeat region. Normally the Cu concentration is about 16-20µM in the blood, 0.5-2.5 µM in the cerebrospinal fluid and about 15 µM in the synaptic cleft. $PRNP^{0/0}$ mice have been shown to have normal Cu-content in the serum, but dramatically reduced Cu-concentrations in the brain and synaptosome (16.2µg/g instead of 193µg/g in the brain). Zn-concentrations did not differ. That Cu is really bound to PrP^C in vivo, has been shown by PrP^C depletion: It greatly reduced Cu content in wild type, but not in $PRNP^{0/0}$ mice (Brown

et al., 1997a). The function of this is not fully clear, but there are two kinds of models (Harris, 2003): An *uptake* model proposes that PrP^C binds extracellular copper to deliver it to an acidic endosomal compartment. An *efflux* model proposes that PrP^C binds copper in the ER or golgi apparatus and takes it out of the cell. The PrP^C molecule has been shown to have a very high affinity to bind copper, in a similar range as CuZnSOD (superoxide dismutase) (Thompsett et al., 2005). The four most affine binding sites are in the octarepeat region at the N-terminal. Copper and zinc stimulate PrP^C endocytosis in cultured neuroblastoma cells (Pauly & Harris, 1998). Brown and colleagues (1999) have shown that cellular PrP shows an encymatic activity similar to superoxide dismutase (SOD). Interestingly, they show that PC12 cells are killed by PrP106-126, a toxic artificial peptide consisting of a stretch of PrP, only in the presence of microglia. The authors also review literature showing that *PRNP*$^{0/0}$-mice are more sensitive to both oxidative stress and copper toxicity. Copper promotes enzymatic reactions leading to more ROS (Fenton- and Haber-Weiss-reaction). Therefore Brown and colleagues (1997b) hypothesize that binding of copper to PrP^C may increase its activity as an anti-oxidant. Brown and colleagues (Brown et al., 1999) hypothesize that a loss of extracellular balance of ROS and their products by PrP^{SC} accumulation could be sufficient to cause pathology of prion diseases. It has been shown that in neurodegenerative disorders (like AD, TSEs, PD or LBD) with increased oxidative stress, PrP^C is upregulated (reviewed in Unterberger et al., 2005). PrP^{SC} is not able to bind Cu with a reasonable affinity, probably because of the altered conformation (Thompsett et al., 2005).

1.3.7.5.4 NO-Metabolism

Keshet and colleagues (1999) have shown that scrapie infected mice as well as *PRNP*$^{0/0}$ mice show abnormal distribution of NOS (Nitric Oxide Synthase), an enzyme crucial for NO production. NO, a gaseous free radical, is involved in processes such as development, synaptic plasticity, regeneration, and regulation of transmitter release. Neuronal Nitric Oxide Synthase (nNOS) was not concentrated in the rafts in scrapie infected mice as in *PRNP*$^{0/0}$ mice, which lead to reduced NO generation in these mice, since rafts are probably used to bring together different signal molecules. The authors state that this is the first reported biochemical similarity

of scrapie-infected and *PRNP*$^{0/0}$ mice. Thus this finding gives a rare hint on loss of function.
NO-metabolism could be important in our context, because NO as a retrograde messenger has a role in long term potentiation and therefore probably in memory function.

1.3.7.5.5 Sleep

Tobler and colleagues (1996) have shown a longer activity-sleep cycle in *PRNP*$^{0/0}$ mice in total darkness than in any other mouse strain. In transgenic mice expressing PrP, the length of the activity-sleep cycle was between *PRNP*$^{0/0}$ and wild type mice. In contrast to the wild type animals, *PRNP*$^{0/0}$ mice showed practically no shortening of the cycle in total darkness. In light-dark cycling environments, *PRNP*$^{0/0}$ mice showed more activity in the second half of the night, while wild type animals showed more activity in the first half of the night; total activity per day did not differ. Sleep architecture and reaction to sleep deprivation was also different: *PRNP*$^{0/0}$ mice showed a much stronger increase of slow wave activity after sleep deprivation. The authors mention a possible analogy to familial fatal insomnia, FFI.
Later, the same group found that sleep deprivation in *PRNP*$^{0/0}$ mice leads to a larger increase of slow wave sleep time and that slow wave sleep is more fragmented (Tobler et al., 1997). The authors interpret this as a lower habitual sleep pressure, but also a less stable sleep control (fragmented sleep is known to lead to attentional problems during the day in humans). A further finding was a lower peak frequency of theta waves in *PRNP*$^{0/0}$ mice on a129Ola background, compared to their wild type counterparts (Huber et al., 1999). This finding was paralleled by less exploratory behavior (which is accompanied by hippocampal theta) and more quiet waking phases in the knockout mice. Besides, the knockout mice showed a higher latency in the passive avoidance test despite comparable learning performance.
In a later paper, both effects (theta peak at lower frequency and more slow waves in sleep after sleep deprivation) were shown to occur over the posterior part, but not the anterior part of the brain (Huber et al., 2002). The authors state that these effects could be due to either changed (inhibitory or serotonergic) neurotransmission or changed influence of oxidative stress in these posterior brain areas.

Related to the function in sleep is the idea that PrP^C has a function in cholinergic neurons in general (maybe even in the neuromuscular junction). This idea is based on two observations: Most, but not all, cholinergic neurons contain PrP^C, and the PrP^C aminoacid sequence shows similarities to the sequence of chicken acetylcholine receptor-inducing activity protein (ARIA) (reviewed in Bendheim et al., 1992).

1.3.7.5.6 Neuronal development and plasticity

Possibly, PrP^C could act as a ligand for laminin/the laminin receptor. Rieger and colleagues (1997) have shown that PrP^C binds the 37-kDa laminin receptor precursor (LRP), which is located at the cell surface, at the same binding domain as laminin binds the LRP. Additionally, they have shown that scrapie-infection (probably the PrP^{SC} accumulation) upregulates the LRP. They conclude that the LRP could act as a receptor or coreceptor for PrP^C on mammalian cells (see also Gauczynski et al., 2001). Graner and colleagues (2000) demonstrated that PrP binds laminin and that this interaction is important for neuritogenesis of cultured hippocampal neurons. PrP^C antibodies inhibited cell adhesion. Laser-inactivation of cell surface PrP perturbed laminin-induced differentiation and promoted neurite retraction. These findings are important for learning, since long term potentiation (LTP) has been shown to depend on laminin (Nakagami et al., 2000).
From the observation that in hamster development (P2) but not in adult hamsters, PrP^C is found along fiber bundles, there was the idea that PrP^C could be involved in axonal growth, perhaps as an adhesion protein (Moya et al., 2000). The authors point out that this could also explain the (Colling et al., 1997) finding of abnormous mossy fiber organisation in *PRNP*$^{0/0}$ mice.
Satoh and colleagues (2000) looked for differential gene expression in prion protein deficient as compared to wild type mouse fibroblasts. They found expression of 15 genes reduced, among these genes important for cell proliferation and adhesion (Cyclins, Eps8 receptor tyrosine kinase substrate; Eps8 is an adapter molecule linking receptor tyrosine kinases to intracellular factors). In contrast, the expression of 27 genes, mainly belonging to the insulin-like growth factor-I (IGF-I) pathway, was

increased. The authors conclude from these data that PrP is involved in the organisation of signalling complexes (like PDGF, EGF, Ras, PI3K and NO). Mouillet-Richard and colleagues (1999) found clues that PrP could be involved in neuronal differentiation: PrP is not expressed in progenitor cells, but in differentiated cells. In their study, they found a differential decrease of 5HT-expressing murine 1C11 cells shortly after starting to express the main enzyme for 5HT-production, while there was no similar decrease in cells committed to be NA-producing cells. Later (Mouillet-Richard et al., 2000) they looked for downstream pathways triggered by PrP^C. They used murine 1C11 undifferentiated and differentiated cells and found decreased fyn phosphorylation upon PrP^C antibodies in differentiated cells, but not in undifferentiated cells. Since the laminin-receptor is found on the surface only, and fyn-signalling within the cell, they looked for mediators and found caveolin1α and 1β. Caveolin was found in differentiated cells only. Further experiments showed that caveolin antibodies really stopped fyn activation. Investigation of subcellular localisation of the fyn-activation showed that neurites give virtually the whole signal. Alternatively, fyn phosphorylation could have been triggered by NCAM, an abundant neuronal adhesion molecule from the immunoglobulin superfamily (Cunningham et al., 1987). PrP^C binds NCAM in mouse neuronal cells (Schmitt-Ulms et al., 2001). It has been shown that NCAM antibodies weaken LTP amplitude (Lüthi et al., 1994). Influences of the tyrosin kinase fyn on memory functions were found by Grant and colleagues (1992). Ca^{2+} influx in the postsynaptic cell after LTP activates a cascade of different protein kinases, including tyrosine kinases. This cascade leads to a release of retrograde messengers, which are thought to enhance transmitter release from the presynaptic cell. Inhibitors of tyrosine kinases block LTP, but they are not specific enough to show which tyrosine kinases are most important for LTP. Grant and colleagues constructed knockout mice lacking one of four nonreceptor tyrosine kinases, which all are expressed in the adult mouse hippocampus, but found effects on memory just in *FYN*$^-$ mice. These mice showed a higher threshold for LTP induction despite normal synaptic transmission (EPSP amplitudes, paired pulse facilitation). There was no effect in NMDA-receptor function as tested with voltage clamp. The authors suppose that the higher LTP threshold could go with lower Ca^{2+}-levels, since lower Ca^{2+}-levels have been observed in T-cells of *FYN*$^-$ mice. Possibly, NMDA- Ca^{2+} is not enough to properly trigger LTP, but also Ca^{2+} from internal stores. They also showed more cells and an abnormal morphology in CA3. On the

behavioral level, they showed practically abolished spatial learning in the Morris water maze (see Figure 21) despite normal single cue learning. In test trials, *FYN*$^{-}$ mice spent not more than ¼ of the time in the target quadrant (chance level).

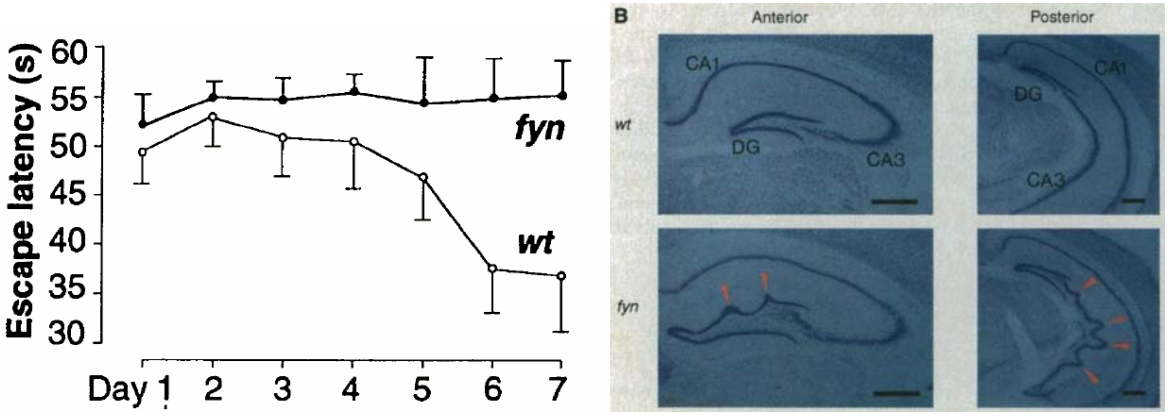

Figure 21: escape latencies over learning runs on consecutive days in the Morris water maze (from Grant et al., 1992) and abnormal cross-sections in fyn^{-}- compared to wild type mice

Schneider and colleagues (2003) showed an influence of PrP^{C} over PKC on NADPH oxidase, MEK (Mitogen-activated protein kinase kinase) and ERK 1/2 (Extracellular Regulated Kinases 1/2), which are important for reactive oxygen species (ROS) production and cell-redox homeostasis in general. In differentiated neurons, this pathway was triggered by caveolin and fyn, but there was also another pathway acting on ERK 1/2, possibly over Grb2->ras-> raf on MEK and ERK 1/2 (Figure 22). The ERK's have been shown to be involved in important functions as cell survival and proliferation.

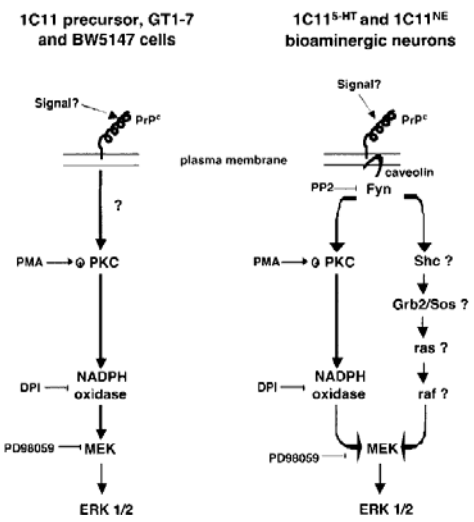

Figure 22: Pathways triggered by PrP^C in nonneuronal and neuronal undifferentiated (left) and neuronal (right) cells; (from Schneider et al., 2003)

Kanaani and colleagues (2005) have shown an effect of recombinant prion protein recPrP on embryonic rat hippocampal neurons in vitro. There was a five-fold increase in axonal length and a 4.6-fold increase in synaptic-like contacts after 7 days of exposure to recPrP, an artificially refolded PrP similar to PrP^C (high α-helix-content). Inhibitors of protein kinase C (PKC) and of Src kinases (e.g. p59Fyn) blocked the effect of recPrP on axon elongation, while inhibitors of PI3-kinase showed a partial inhibition. This could mean that these pathways are responsible for the action of PrP on axonal growth. The effect was specific in that N- or C-terminal parts or the Dpl-protein did not have an effect.
Steele and colleagues (Steele et al., 2006) looked for neuronal development in proliferating zones (dentate gyrus in hippocampus and subventricular zone) in cells differing in PrP^C expression (*PRNP*$^{0/0}$, wild type and overexpressing cells) and found rising concentrations in adult cells compared to progenitor cells. Cells overexpressing PrP^C showed earlier differentiation and proliferation, but in the end cell numbers were comparable between *PRNP*$^{0/0}$, wt and overexpressing cells. The authors conclude that PrP^C is a sort of 'commitment switch' for neurons.

A new paper could possibly link several functions reviewed here: Nieznanski and colleagues (2006) found that cellular PrP is able to inhibit tubulin oligomerization, probably by direct binding (Nieznanski et al., 2005). Tubulin is main component of microtubules. Build-up and destruction of microtubules by polymerisation and

oligomerisation is a sensitive process (Nogales & Wang, 2006), which is very important for synaptic plasticity, and possibly late LTP.

1.3.7.5.7 Neurophysiology and Learning

Collinge and colleagues (1994) recorded electrical activity of hippocampal cells from normal and *PRNP*$^{0/0}$ mice and found weakened $GABA_A$-receptor mediated IPSP and impaired long-term potentiation. The EPSP amplitude in null mice decreased very fast. The authors propose that abnormal clustering of $GABA_A$-receptors (farther from the synapse) lead to abnormal IPSP, which lead to NMDA-receptor overactivation, which impairs normal LTP.
Manson and colleagues (1995) have confirmed impaired or abolished LTP in *PRNP*$^{0/0}$ mice and even shown a gene dose effect, in that heterozyguous *PRNP*$^{+/-}$ mice, which have reduced PrP^C in the brain, show also an LTP impairment with weak LTP or even only “short time potentiation” (STP, Figure 23).

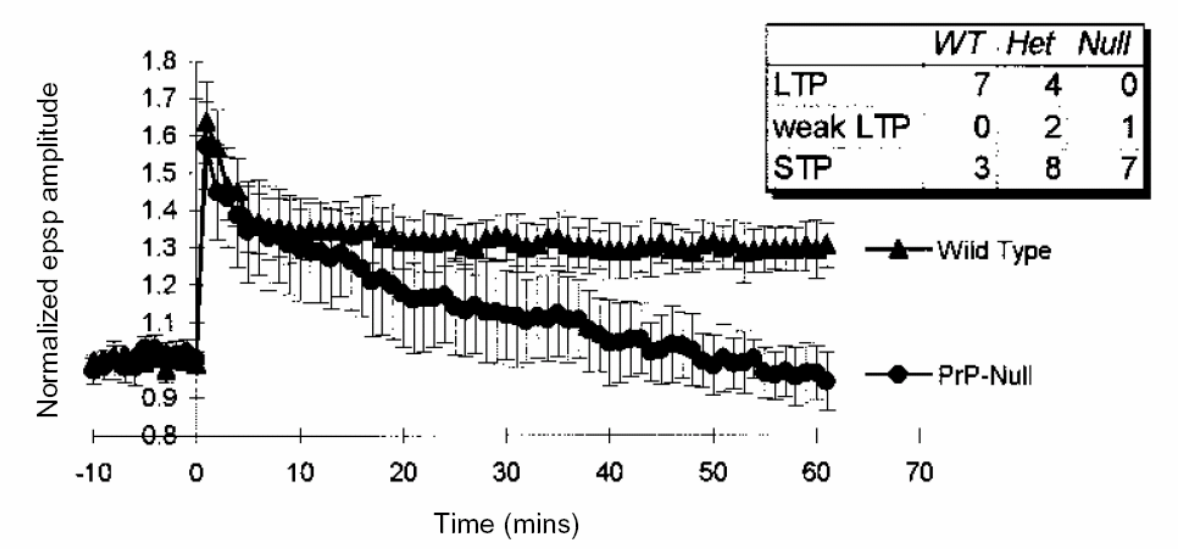

Figure 23: Impaired LTP in *PRNP*$^{0/0}$ mice; the table shows impairment even in heterozygous mice (from Manson et al., 1995)

Whittington and colleagues (1995) have shown that this deficit can be rescued with an appropriate concentration of a human PrP transgene.

Other groups did not find any electrophysiological differences between wild type and homozygous *PRNP*$^{0/0}$ mice (Herms et al., 1995 [Purkinje cells]; Lledo et al., 1996 [CA1]).

Another study showed different granulation and mossy fiber sprouting in gyrus dentatus and CA3 in *PRNP*$^{0/0}$ mice, combined with smaller electrophysiological excitability in terms of lacking superimposed population spikes (Colling et al., 1997). The authors explain the smaller excitability with a downregulation to prevent epileptic activity, which could arise of the unfavorable cellular architecture similar to the architecture seen in human epileptics or in kainic acid lesion models of epilepsy in animals.

Mallucci and colleagues (2002) constructed conditional *PRNP*$^{0/0}$ mice to test effects of post-birth knockout on neurophysiological measures. The mice stopped to produce PrP 10 weeks after birth and were greatly normal. Like *PRNP*$^{0/0}$ mice, they showed reduced slow and medium after-hyperpolarisation (AHP) in CA_3. A PrP transgene rescued this phenotype. In both types of mice, there were no signs of neurodegeneration. As a mechanism, they propose a PrP influence on fyn-signalling (see below or Mouillet-Richard, 2000), which could influence Ca^{2+}-dependent K-channels to reduce after-hyperpolarisation. The authors state that these neurophysiological differences are similar to those observed in scrapie. However, loss of normal function of PrP^C does not seem to be the reason for the disease.

Maglio and colleagues (2004) found a lower threshold for LTP in dentate gyrus preparations of 3-4 months old mice. By in situ localisation, they found a higher expression of NMDA NR2A and NR2B receptor subunits, which could possibly account for the higher excitability of the dentate gyrus neurons. In a recent paper (Maglio et al., 2006), they confirmed a lower threshold for LTP generation in aged (9 month old) mice. In addition, LTP persisted longer in aged knockout mice than in aged control mice. The authors state that this gives a hint that prion protein could serve the regulation of synaptic transmission, and that a normal downregulation during aging does not take place in knockout mice. They say that a higher plasticity in aged mice not necessarily parallels better learning, since higher excitability could lead to a saturation effect. Besides, the higher excitability could result from an impaired GABA-ergic inhibition (see Collinge et al., 1994).

In a study with mice of two different genetic backgrounds, a selective spatial learning deficit could be rescued by introduction of hamster PrP (Criado et al., 2005). On a

neurophysiological level, they found a weaker paired-pulse facilitation and LTP. Both effects could be at least partly restored with hamster PrP.

1.3.7.6 The Met129Val Allelic Variant

There are two variants per allele: Methionine (Met, coded by ATG) and Valine (Val, coded by GTG). 129Met is the ancestral allele, 129Val is found in humans exclusively (Schaetzl et al., 1995). The polymorphism is found in a short β-sheet (Schätzl, 2001). The normal population has a frequency of 37% Met/Met, 51% Val/Met and 12% Val/Val (Palmer et al., 1991).
Studies have shown that the Met-allele has a higher propensity to form β-sheet structures in artificial peptides (Petchanikow et al., 2001) and in the native human protein (Tahiri-Alaoui et al., 2004).
In a large sample, Val/Val carriers had lower MMSE scores at the age of 65 years, compared to age- and gender-matched Val/Met or Met/Met-groups (Berr et al., 1998). Val/Met versus Met/Met-carriers did not differ. The effect was similar like the effect of APOE ε4 and was independent from the APOE-effect. The authors do not list differences in subtests. A similar effect was found in another large-sample study (Croes et al., 2003): Homozygous Val/Val carriers showed earlier cognitive decline in MMSE scores, namely at an age around 60. At later age (after 65), cognitive declines were comparable among genotypes.
Rujescu and colleagues (2002) report reduced whole brain white matter volumes and larger volumes in schizophrenic or healthy Met/Met carriers relative to Val/Met or Val/Val carriers. However, they do not list data on the healthy subjects alone. Besides, Met/Met carriers were significantly older than the Val/Met-Val/Val group.
In a large randomly selected German sample (n=335), Rujescu and colleagues (2003) found higher scores of Val/Val carriers than Val/Met or Met/Met carriers in HAWIE intelligence scores (Val/Val: 117.4(SD14.4); Val/Met: 112.2(14.1); Met/Met: 110.9(15.2)). These came from broad advantages in verbal and performance subtests, most prominently in the digit symbol test, which relies on working memory and executive function.

Differential expression:

Garmy and colleagues (2006) have observed differential expression of human valPrP^{C} or metPrP^{C} in intestinal epithelial cell lines. For example, in HT-29 cells, only metPrP^{C} RNA was found (the authors do not mention how this would be possible in Val/Val homozygous individuals). The authors stress the importance of their findings for disease, since cells containing the Met allele seem to be required for the generation of infectious prions (Wadsworth et al., 2004), and intestinal epithelial cells could be a first target for infection with PrP^{SC} (Morel et al., 2005), so these cells could promote prion spreading.

Influence on memory:

Papassotiropoulos and colleagues (Papassotiropoulos et al., 2005) found better memory for words in a 24h-recall in young, healthy carriers of at least one 129Met allele (129MM: n=159 and 129MV: n=151) compared to 129VV carriers (n=44), but not in immediate or 5min recall. This result was observed in two independent (academic vs non-academic) groups. The paper in the center of this dissertation tried to replicate this finding in an indirect way with fMRI, to constrain more precisely when it starts, and to find hints where it comes from.

Article

2 Prion Protein M129V Genotype and Memory-Related Brain Activation: An Event-Related fMRI Study

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Abstract

There are large inter-individual differences in memory abilities. A considerable part of these differences could be explained by genetic variability. An earlier behavioral study (Papassotiropoulos et al., 2005) has shown that the prion protein Met129Val polymorphism interferes with word retrieval after a 24 hours time lag. We tried to replicate this finding in an event related fMRI study with three genotypic groups (Met/Met, Val/Met, Val/Val), which were carefully matched for sex, age, education, other memory-related genes and performance in episodic memory. We found greater fMRI activation during word recognition in Val-carriers compared to Met-carriers both 30 minutes and 24 hours after learning of a word list, despite matched performance. In addition, correlations between fMRI activations and recognition performance were rather positive in Met-carriers and rather negative in Val-carriers. We interpret our results as a lesser memory performance in Val-carriers than in Met-carriers. Independently from the fMRI results, we found higher grey matter densities in wide parts of the brain in Val-carriers. These differences point to a role not only of the prion protein, but also of the Met129Val polymorphism in neuronal plasticity at a time-scale of minutes to hours.

Introduction

While estimations from twin studies indicate that about 50% of individual differences in memory performance can be explained by genetic factors (McClearn et al., 1997), little is known about which genes influence memory in healthy individuals. Probably effects of single genes are very small, and virtually any genetic variant involved in

CNS function could lead to differences in memory performance. From about 3-6 million single base pairs differing within the human genome ('Single Nucleotide Polymorphisms' or SNP's), some 100000 are estimated to influence CNS functions (Goldberg and Weinberger, 2004). Another problem is that found associations can not directly be proven to be causal in humans, since polymorphisms can be linked to other polymorphisms ('linkage disequilibrium'), so it can hardly be decided which variant confers the measured difference. Goldberg and Weinberger (2004) propose strategies to circumvent these problems: (1) Knowledge-based selection of few candidate genes instead of testing every gene; (2) replicating results with different methods or accounts and (3) confirming results with independent populations. Studies using this research strategy isolated important genes in two different domains: Genes directly involved in neurophysiology (for example the gene encoding the serotonin 2A receptor; de Quervain et al., 2003) or genes involved in diseases affecting the CNS (for example the apolipoprotein E gene, which is a risk gene for Alzheimer's Disease; Bookheimer et al., 2000).

The actual study investigated the Prion Protein Gene (*PRNP*) involved in all mammal prion diseases like Bovine Spongiform Encephalopathy (BSE) in cows or Creutzfeldt-Jakob Disease (CJD) in humans. The infective agent, the 'prion' (Prusiner, 1982) has practically the same aminoacid sequence as a cellular gene (the *PRNP* gene) (Oesch et al., 1985). Its product, the prion protein (PrP) is found predominantly in synaptic membranes (Moya et al., 2000) throughout the brain, at the highest concentrations in the hippocampus, septal nuclei and in the nucleus caudatus (Bendheim et al., 1992). In some cells prion protein is also found in the cytosol, but it is unclear in what kinds of cells and why (Mironov et al., 2003). However the normal protein, termed PrP^{C} (C for 'cellular') and the infective protein, PrP^{SC} (SC for 'scrapie') differ in their secondary structure: PrP^{C} consists of about 40% α-helix and little β-sheet, PrP^{SC} of

about 30 % α-helix and 45% β-sheet (Prusiner, 1998). PrP^{SC} seems to be the dominant form and to replicate by refolding PrP^{C} into PrP^{SC}. As a consequence, PrP^{SC} is much more prone to aggregate to amyloid structures. The influence of this differential folding on the pathologic process is not understood yet.

Studies in Aplysia have shown that a similarly switching protein, the 'cytoplasmic polyadenylation element binding protein (CPEB)' helps to stabilize long term memories ('long term facilitation' (LTF); Si et al., 2003a, b). In vitro, only CPEB aggregates were able to bind CPE, while the soluble form was not. CPEB has also been found in humans. Shorter and Lindquist (2005) point out that this mechanism could nicely explain how molecular memory traces can persist longer than the molecules involved. However to our knowledge there are no data about this mechanism in other species than Aplysia.

Studies with *PRNP* knockout mice also point on an influence of PrP on memory functions: Lacking PrP strongly shortens long term potentiation (LTP; Collinge et al., 1994; Manson et al., 1995; Whittington et al., 1995; Criado et al., 2005; but see Herms et al., 1995; Lledo et al., 1995), the cellular basis of long term memory (Whitlock et al., 2006; Pastalkova et al., 2006). Maglio and colleagues (2004; 2006) found a lower threshold for LTP induction in *PRNP* knockout mice, which was probably due to differential NMDA receptor subunits composition. One study found a spatial learning deficit in *PRNP* knockout mice, which could be rescued with hamster PrP (Criado et al., 2005).

In young, healthy humans a common ATG (Met) to GTG (Val) substitution at codon 129 has been shown to influence long term memory: In two large independent samples (academics and non-academics), Papassotiropoulos and coworkers (2005) showed a lower performance of Val/Val carriers in free recall of words 24 hours after learning, with comparable performance at immediate recall and recall after 5 minutes.

We planned the present study to replicate and refine this finding with fMRI and to learn whether different brain networks are involved during recognition of single words learned 30 minutes or 24 hours ago. We introduced a new interval of 30 minutes to determine if the genetic differences have rather to do with overnight consolidation effects or with classical late LTP. Since studies showed that LTP faded in this time-scale in knockout mice (see e.g. Collinge et al., 1994), we expected to see differences in recognition-related fMRI activation already in the 30 minutes condition. We followed the logic of earlier studies (Bookheimer et al., 2000; Bondi et al., 2005; Mondadori et al., 2006) to match memory performance between groups and to interpret differences in recognition-related fMRI activation in terms of cognitive effort to reach the same memory performance (memory performance was not treated as a dependent variable). This led us to the following hypotheses about carriers of the different Met/Met Val/Met and Val/Val combinations:

(1) the more Val alleles, the more activation during word recognition

(2) the more Val alleles, the more negative correlations and less positive correlations between fMRI activation and performance level within group.

(3) because the prion protein is expressed strongest in hippocampus, striatum, septum and olfactory bulb, but less in frontal/ parietal areas (Bendheim et al., 1992), we do not expect significant activation differences in a working memory task

(4) standardized neuropsychological tests of memory (which has been matched in our groups), verbal abilities, intelligence and executive functions do not differ between groups of young prion 129 Met/Met Val/Met and Val/Val genotype carriers

(5) local differences in grey matter concentration or whole brain volume differences could be found, since differences in the structure of single brain areas have been observed in knockout mice (in the dentate gyrus and CA3 of the hippocampus: Colling et al., 1997) and prion protein has been found to be expressed widely during

axonal growth (Salès et al., 2002), which could influence the global brain structure; however, we are not able to predict the direction of possible differences, because we do not know if or how the Val or Met alleles differentially influence neuronal growth.

Methods

Subjects

From 46 subjects who passed the fMRI experiment, 5 had to be excluded (2 because of too much motion in the scanner, 3 because of excessive repetition of the words learned on day 1). From the 41 remaining subjects, 36 were chosen for the study, yielding equal groups of 12 subject of each genotype (Met/Met, Val/Met, Val/Val; mean age 23.2 (SD 2.0) years; 7 female in each group). The subjects reported no psychiatric or neurological problems and denied taking illegal drugs or prescription medication. All subjects gave written informed consent to participate in the study. The experiments were approved by the ethics committee of the Kanton Zurich.

Matching

Groups were matched for age, years of education, apolipoprotein E (APOE) genotype and serotonin-2a receptor genotype (de Quervain et al., 2003), Kibra genotype (Papassotiropoulos et al., submitted) and behavioral performance in the episodic memory experiment in terms of number of correct 'Remember' answers in the 24h condition (Table 1). Behavioral performance in the fMRI task is not treated as a dependent variable of the study, and imaging data were evaluated only after having completed the matching procedure. Performance matching is important since activation depends on performance (McDermott et al., 2000; Meltzer and Constable, 2005), and we did not want results to be confounded with performance effects.

Experimental Procedure

Subjects underwent a neuropsychological examination in advance of the main experiments. The experimental session was conducted on two consecutive days at the same time of day (Figure 1). On day 1, subjects had to learn a first list of words, followed by a free recall; the rest of the experiment was conducted on day 2. On day 2, subjects first learned a second word list. Then they practiced the working memory task. Then they moved into the scanner. After the survey images, they were instructed about the recognition task. Then they underwent the recognition task, in five big time-series of 7 min 15 s. Then they underwent the working memory task. In the end of the session, the anatomical images were obtained. Total scanning time was 59 minutes plus brakes. After moving out of the scanner, subjects had to answer a structured interview.

fMRI Experiment on Word – Recognition

Subjects had to learn two lists of 50 verbs with the instruction to form a sentence with autobiographical content for each verb. The two lists were learned 24h 30min (day 1) and 30min (day 2) before the recognition test. To match the percentages of correctly recognized words between time lags, an additional instruction was administered on day 1: The experimenter read the sentences to the subjects, giving only the first two phonemes of the verb, and the subjects had to retrieve the verb. If they did not respond within 3 seconds, the experimenter said the verb. After the learning phase, subjects had to recall as many verbs as possible within 5 minutes. Subjects were not told that they had to remember the learned verbs after the free recall to avoid rehearsal between study-phase and test-phase (this was checked in the structured interview).

The total of 300 items (50 old words learned 24 hours ago, 50 words learned 30 minutes ago, 100 foils, 50 letter strings, 50 null trials) were presented in 6 time-series of 50 items in pseudorandom sequence (same sequence for all subjects) in a fast event related design. Mean inter stimulus interval was 8.82s, with a pseudo-random jittering of up to 2.5s to safely sample the whole length of the hemodynamic response function. Stimuli were presented with Presentation (http://nbs.neuro-bs.com).
Each trial started with the word or letter string, presented for 2000ms, followed by a fixation cross for 6820ms plus or minus the pseudorandom jittering of up to 2.5s (range: 4320 to 9320ms). Each time series took about 7 min 15 s. To control for the liveliness of the recollection of the verbs, we used Tulving's Remember / Know operationalisation (Tulving, 1978): Subjects were instructed to indicate recognition in terms of 'Remember' (full, lively recollection including context knowledge), 'Know' (mere familiarity) or 'New' (Tulving, 1985). For the nonwords, subjects had to compare the first and the last letter and to indicate 'Identical' for same letter in same case (e.g. bxxxb or BxxxB), 'Same' for same letter with different cases (Bxxxb) or 'Different' for different letters (Bxxxc or bxxxc). Responses were collected with a response box that subjects held in their dominant hand.

fMRI Experiment on Working Memory

The experiment included one fMRI time-series with a 2-back task for the assessment of working memory and a baseline task ('x-target'). Stimuli were presented block-wise, in 5 alternating blocks of 13 stimuli per condition. Blocks were announced by an instruction slide. Instruction slides were shown for 3s, stimuli for 1.845s, adding to 27s per block. The 2-back task required subjects to respond to a letter repeat with one intervening letter (e.g. S – f – **s** – g). The 'x-target' task required subjects to respond to each letter 'x' (Mondadori et al., submitted).

Materials

Stimuli were concrete, frequent, easily imaginable verbs. Most of them were drawn from a German handbook of word norms (Hager and Hasselhorn, 1994). From these verbs we built 4 lists of 50 verbs carefully matched for word length, semantic content, and normative ratings of imaginability, concreteness and valence. These 4 lists were used for all subjects. The lists learned on day1 / day2 were counterbalanced to avoid list effects on the delay conditions. Nonwords were 100 consonant strings matched to the verbs in length. Stimuli were written white on black in Arial bold 72pt letters (the letters covered an angle of 0.87x0.65°). For the working memory experiment, stimuli were 50 upper- or lowercase letters typed in black on a white background (viewing angle 4.4x3.3°).

MRI Data acquisition

MR measurements were performed on a 3T Philips Intera whole body MR scanner equipped with an eight-channel Philips SENSE head coil. Functional data were obtained from 32 transverse slices (without interslice gaps) parallel to the AC-PC plane covering the whole brain with a measured spatial resolution of 2.8 x 2.8 x 4 mm^3 (acquisition matrix 80 x 80) and a reconstructed resolution of 1.7 x 1.7 x 4 mm^3. Data were acquired using a SENSE-sshEPI (Schmidt et al., 2005) sequence with an acceleration factor of R = 2.0. Other scan parameters were TE = 35 ms, TR = 3000 ms, θ = 82°. A standard 3D T1-weighted scan was obtained for anatomical reference with a measured spatial resolution of 1 x 1 x 1.5 mm^3 (acquisition matrix 224 x 224) and a reconstructed resolution of 0.9 x 0.9 x 0.8 mm^3, TE = 2.3 ms, TR = 20 ms, θ = 20°.

fMRI Data Analysis

Image pre- and postprocessing and the statistical analyses were performed with SPM2 (http://www.fil.ion.ucl.ac.uk/spm). Standard preprocessing procedures were applied, realignment, normalization and spatial smoothing (7 mm Gaussian kernel) (Friston et al., 1995a). On the single subject level, data were analyzed according to the fixed effects model (SPM2). The six head movement parameters were included in the model as confounding factors. Data were high-pass filtered with a standard filter-value of 128s, which corresponded nicely to the rule of thumb '2 times maximal stimulus onset asynchrony times TR'. On the second level, within-subject contrasts were entered into random effects analyses (ANOVAs and T-tests, SPM2) which account for variance between subjects (Friston et al., 1995b). We also computed correlations between the within-subject recognition contrasts and behavioral measures (simple regression, SPM2). Thresholds were set at a $p < 0.001$ level, uncorrected for multiple comparisons. We chose an extend threshold of 12 voxels, with the exception of the hippocampus, where we report also smaller activations of at least 5 voxels (because of the high a priori probability to be involved in episodic memory, and the small volume of this structure).

Anatomical MRI Data Analysis

Based on the 3D-T1-weighted structural MRI images, group differences were computed with optimized voxel based morphometry (VBM) (Ashburner and Friston, 2000; Good et al., 2001) with a Matlab-Script in SPM2 (http://dbm.neuro.uni-jena.de/vbm.html), which compares voxel-wise comparisons of grey-, white-matter or liquor probabilities between groups. The procedure yields whole-brain grey-, white-matter and liquor volumes as a by-product.

ANOVAs with PRNP genotype as independent variable and head size as covariate were computed to determine group differences in brain volumes. Thresholds were set at $p<.05$ for whole brain data and at $p < 0.001$ for probability maps (considered were only spots of 100 voxels or more to reduce false positives; analogous to the minimal volume of 12 voxels in functional data; for the hippocampus, 40 voxels or more), not corrected for multiple comparisons. Head size was considered as a covariate of no interest (ANCOVAs) to avoid possible bias for head sizes in the normalization process used in the VBM procedure.

Neuropsychology

Memory functions were assessed with the “Verbaler Lern- und Merkfähigkeitstest” (VLMT, German version of Rey Auditory Verbal Learning Test; Helmstädter et al., 2001), and the subtests “visuelle Paarerkennung” (visual pair associate) and “verbale Paarerkennung” (verbal pair associate) of the Wechsler Memory Scale Revised (Härting et al., 2000), German version. Intelligence was measured with the subtests “Zahlennachsprechen” (digit span), mosaic test and “Gemeinsamkeiten finden” (abstract thinking) of the Hamburg Wechsler Intelligence Test (Tewes, 1991), and the vocabulary test MWT-B (Mehrfachwahl-Wortschatz-Intelligenztest; Lehrl, 1999). Executive functions were assessed with the Stroop Test (Stroop, 1935), which measures the suppression of interference. Data were evaluated with ANOVAs and T-Tests. Thresholds were set at $p<.05$, not corrected for multiple comparisons.

Results

Neuropsychology

The performance-matched groups did not differ significantly in all standardized neuropsychological tests of memory, verbal and figural intelligence, vocabulary and executive function (Table 2).

Performance in fMRI Tasks

The genotype groups did not differ significantly in performance, neither in number of correct answers, nor in reaction times (Table 3). This held true not only for number of correct 'Remember'-answers in the 24h recognition task (matching), but also for free recall and 30min recognition task. Numbers of 'know' answers misses and false alarms were relatively low, but also comparable between groups. Performance between time intervals (30min/ 24h) was comparable (e.g. Correct Remember answers p=.528 in the sample pooled for genotypes; n=36).

The baseline task showed a roof effect (most of the subject reached the maximum); reaction times did not differ significantly as well. The same held true for the baseline of the working memory task ('x-target'). Performance in the working memory ('2-back') task did not differ significantly between genotype groups.

fMRI Results: Pooled Sample

In the recognition task, the event-related design allowed us to differentiate between activations in correctly recognized items from activations in falsely recognized items (false alarms). The study was designed to obtain a high number of 'remember' answers. This allowed us to have a purer measure of episodic memory. On the other hand, there were not enough 'know' answers to be analyzed separately, so we included only 'remember' answers in the evaluated contrasts. One-sample t-tests

with the whole sample (n=36) yielded reasonable activation patterns of the recognition conditions over the low-level letter baseline task: Activation in recognition (both ‘Remember 30 min ’ and ‘Remember 24 h’) trials significantly exceeded the letter string baseline task in frontal areas (Brodmann’s Areas BA 47, 10, 9, 8 and 6), caudate, middle temporal gyrus, angular/supramarginal gyrus and posterior cingulate, predominantly on the left side (due to the verbal nature of the task). In the inverted contrast, activation spots were located in frontal areas (BA 44, 45, 46 and 6), the thalamus, in all temporal gyri, insula, parietal areas (BA 1 and 7) and in the inferior occipital gyrus (BA19). Frontal activations were often right-lateralized, the rest spread on both hemispheres.

With false alarms as a higher-level baseline, activations were much more symmetrical to the midline (Tab. 4). Frontal activations were confined to the dorsolateral prefrontal cortex (BA 8, 46), because the contrast reflects retrieval success. There was a more wide-spread activity in the temporal lobes bilaterally, including the parahippocampal gyrus (BA 28 right, 36 left). There were activations in the angular/ supramarginal gyri on both sides (BA 39, 40). Inverted contrasts showed practically no activations, except the inferior frontal gyrus (BA 11 right) if compared to the 24h-recognition condition, probably showing a higher retrieval effort in items misclassified as old.

The working memory task (2-back task) compared to a simple ‘x-target’ baseline showed activation pattern in a fronto-parietal network (BA 47 and 7 bilaterally, BA 10 and 19 left; see Table 3, Figure 1), the inverted contrast showed activations in BA 11 left, insulae bilaterally, the parahippocampal gyrus bilaterally and precentral and postcentral gyrus. The parahippocampal activations in the very easy baseline task relative to the more demanding working memory task could be seen as a ‘wandering

mind' phenomenon of the subject freely remembering things that are not demanded by the task (Stark and Squire, 2001).

fMRI Results: Genotype Effects

We calculated ANOVAs between genotype groups (Table 5, Figure 2). ANCOVAs with performance (in terms of hits minus false alarms) as a covariate yielded qualitatively similar results (not shown). Areas activating in relation to recognition success can be seen in the correlations (supplementary Table S2, Figure 3). Carriers of one respectively two Val alleles consistently showed more activation in a dose-dependent manner (Val/Val > Val/Met > Met/Met; Table 5, Figure 2). This effect can be seen in both the 30min and 24h conditions (slightly more consistent in the 30min condition) and with both false alarms and the letter task as baseline. There was no evident localisation of these differences; they were situated in all areas involved in retrieval effort or retrieval success. During False Alarms, Met carriers activated more than Val carriers, also in a dose-dependent manner. Most of these activations were situated in the right hemisphere. Comparisons between homozygous (Met/Met and Val/Val) versus heterozygous (Val/Met) carriers showed few significant differences, so the effects have to be seen as the additive influences of the Val-allele rather than effects of homozygosity versus heterozygosity. Correlations of retrieval success activation (Remember-answers over False Alarms) with recognition performance showed an impressive dissociation: While Met/Met and Val/Met carriers showed either no or positive correlating spots (more prominently 30min after learning), Val/Val carriers showed far more negative correlations, which means the higher the activation of a subject in such a region, the worse the performance compared to other persons of the same genotype group (supplementary Table S2, Figure 3).

In the working memory task, Val/Met carriers showed slightly more activations in the right frontal / temporal lobe than the two extreme groups (Table 5). This group showed also more positive correlations of activation with retrieval success (supplementary Table S2).

sMRI Results: Genotype Effects

In our sample, whole brain volumes for grey matter, white matter and cerebrospinal fluid were comparable between prion protein subgroups, with a small tendency for smaller brains in Val/Val carriers (Table 6).

We also compared local maps of grey matter probabilities (Voxel Based Morphometry (VBM); Ashburner and Friston, 2000) between genotype groups. We chose parameters as closely analogous as possible to the functional ones. ANCOVAs with head size as covariate (ANOVAs yielded very similar results, data not shown) showed higher grey matter probabilities in Val carriers in wide parts of the brain, in a dose-dependent manner. This seems to go parallel with the functional MRI results. However, the most significant spots are situated in very different areas (Figure 4). To control for the influence of such partial volume effects, we conducted voxel-wise ANCOVAs with grey matter densities as covariate (BPM: Casanova, 2006). Some activation spots changed, but the overall pattern of Val overactivation relative to Met carriers persisted (data not shown).

Discussion

Exploration of the influences of single genes or genetic polymorphisms on a cognitive function goes in two steps (Ramus, 2006): (1) Candidate genes have to be found and replicated by showing that carriers (humans or animals) of different genetic variants

differ systematically in the function of interest. This is called the ‘top-down’ step, because it leads from the level of a whole brain or organism to the genetic level. (2) It has to be shown how the genetic variant influences the function, which is much more difficult, because it leads us ‘bottom-up’ through several levels from the gene over protein expression levels and cellular functions and functions of whole organs to the level of the whole brain or organism.

In our example, the study of Papassotiropoulos and colleagues (2005) was dedicated to the first step: It found a lesser performance in *PRNP* M129V carriers in word retrieval 24 hours after learning, but not five minutes after learning. This result pattern was found in two independent samples (students and trainees) containing 240 and 114 individuals. The actual study had two aims: First, we wanted to replicate the behavioral results by different means, and second, we looked out for potential mechanisms using functional MRI and structural MRI and broader neuropsychological measures than in the Papassotiropoulos’ (2005) study. In addition, introducing a new 30 minutes retrieval condition should help to narrow the time window of the effect. The replication belongs to step (1) cited above, but the study can possibly also say something about the mechanisms (2), since brain imaging may be nearer to genetics than behavior.

Hypotheses (1) and (2) cited in the introduction are a translation of the behavioral findings of the Papassotiropoulos’ et al study into an fMRI setting. We followed the logic of earlier studies (Bookheimer et al., 2000; Bondi et al., 2005; Mondadori et al., 2006) and compared fMRI activations during memory tasks (in our case, word recognition) in genetic groups with matched performance in the task. Consequently, we matched not only for performance and other genes implicated in memory processes (Table 1), but tried also to control for the phenomenological quality of the recognition by using Tulving’s Remember-Know differentiation (Tulving, 1972), which

introduces a criterion for the liveliness of a memory representation, namely if contextual features of the situation can be retrieved, which is a feature of episodic as compared to semantic memory. Under this condition, we interpret (1) stronger and more wide-spread fMRI activation during recognition of words and (2) more areas with negative instead than positive correlations between fMRI activation and recognition performance in terms of more difficulty of a person to reach the same memory performance level, and therefore less capacities of his/her memory system. Using this strategy, we found a clear cut result pattern: During recognition of words learned both 30 minutes or 24 hours 30 minutes earlier, (1) individuals with more Val alleles of the PRNP Met129Val polymorphism showed more activity than individuals with less Val alleles (i.e. more Met alleles; Figure 2; Table 5) and (2) individuals with more Val alleles showed more negative correlations and less positive correlations between fMRI activation and recognition performance (hits minus false alarms; Figure 3; supplementary Table S2) than individuals with less Val alleles. We interpret these additional increases in blood oxygenation in Val carriers, which are not associated with increased, but with reduced recognition performance, in terms of an unsuccessful effort to overcome weaker memory skills (Figure 5). In this light, our study replicates the cited behavioral findings in two large samples with different methodology. However, our effect gets continuously stronger with rising number of Val alleles and is not only found in homozygous Val/Val carriers.

Given this finding of reduced memory performance of *PRNP* 129 Val carriers as compared to Met carriers, we now try to speculate about the mechanisms in the light of the actual study and the fast-growing literature on the prion protein. We will do this in four steps: First we will review some findings about the prion protein Met129Val

polymorphism. Second, we will try to choose which biological memory mechanisms are likely to occur in the same time window as our observed results. Third, we will speculate about the functional specificity of the observed genotypic differences. And fourth, we review some additional findings in the light of the actual study.

The Met129Val substitution is the most-studied single nucleotid polymorphism (SNP) of the prion protein gene. 37% of caucasian populations carry the Met/Met-combination, 51% Val/Met and 12% Val/Val (Palmer et al., 1991). Met seems to be the ancestral allele, while Val was found in humans exclusively (Schaetzl et al., 1995). It has been shown that metPrP has a higher propensity to form β-sheets than valPrP (Tahiri-Alaoui et al., 2004), which promotes the formation of amyloid structures. This polymorphism has a profound influence on the risk to develop CJD-symptoms: Heterozygous Val/Met carriers are strongly underrepresented in affected cases (Mead et al., 2001). Studies about differences in healthy carriers of the different alleles are rare: It would be very useful to have experiments with knock-in mice, but to our knowledge such studies do not exist.

We now try to speculate about the mechanisms that could have lead to our results: In our young, healthy sample, pathological mechanisms can probably be ruled out, so we have to look for physiological mechanisms that could influence memory performance. We do not see any evident pattern of the memory-related activations between genotype groups suggesting that carriers of one allele use rather one memory system and carriers of the other allele another. The locations of the activation spots look rather random, while the consistent overactivation of the Val-allele carriers relative to the Met-allele carriers is striking. We have to assume that Val-carriers need more activation in broad parts of the brain to reach the same memory performance as the Met-carriers. Our study and the study of Papassotiropoulos and colleagues (2005) allow to pinpoint the time course of the

differential processes between the genotypic groups: We do not see any hints for differences in learning and recall after five minutes (though, we do not have any imaging data for these time points). So we can assume that the observed differences are not due to different abilities in learning or in language functioning. At the 30 minutes time lag, we see already large differences in activations and in correlations between activation and recognition success within groups, so the difference probably develops in the course of minutes (but is too weak to be seen with behavioral methods after five minutes). After 24 hours, there was a behavioral effect in the large sample study and an at least equal effect as at the 30 minutes condition in the imaging study: Although differences in activation were rather smaller in the 24 hours condition than in the 30 minutes condition (Table 5), the negative correlations between activation in Val/Val carriers and recognition success became stronger over time lags, while Met/Met carriers did not show significantly less positive correlations (Supplementary Table S2). In sum, the prion group differences seem to develop in the time frame of minutes and stay until one day after learning. This nicely fits the electrophysiological data cited in the introduction, which say that measures like LTP are changing after some minutes in *PRNP* knockout mice. Of course our effects are much smaller, but we do not compare humans with or without expressed prion protein, but humans with slightly modified types of prion protein, which could possibly influence enzymatic activity of the protein. The relation between LTP and hippocampal learning has been assumed for a long time and shown quite convincingly recently (Whitlock et al., 2006; Pastalkova et al., 2006). Although there are not yet any cell-level data comparing cells expressing metPrP with valPrP (which is not found in mice), data involving prion protein in cellular plasticity are accumulating: PrP binds laminin (Rieger et al., 1997) and NCAM (Schmitt-Ulms et al., 2001), molecules important for LTP (Nakagami et al., 2000, Luethi et al., 1994); it is

involved in NO-metabolism (Keshet et al., 1999), which is also important for LTP (see e.g. Miyamoto, 2006); and it has even been shown to influence tubulin oligomerization in vitro, a process important for microtubule formation and neuronal plasticity (Nieznanski et al., 2006).

Given our prion protein gene group difference in single word learning, we should ask ourselves how specific these effects are. For comparison, we conducted a working-memory task (2-back task) and compared results between the same genotype groups. There were only few differences in brain activations (Table 5), but more positive correlations between task activation and working memory success in Val/Met carriers than in the other groups (supplementary Table S2). This means that hypothesis 3 is incorrect. At any rate, the pattern is fundamentally different from the pattern in the episodic memory task (no gene dose effect, no negative correlation in the group with most activation spots). Are there other hints on different working memory performance in Val/Met carriers? In the neuropsychological paper-and-pencil tests, we assessed verbal memory, visual memory, different intelligence measures and one executive function. There were no significant differences in ANOVA's between genotypic groups in all of these tests (Table 2). This means that hypothesis 4 turns out to be true. However, in other populations, which were older and not matched for memory performance (which is often positively correlated with intelligence), there were genotypic differences: In a normal elderly population, Val/Val carriers performed worse than the two other genotypic groups in the mini-mental status score, which consists of orientation, visuoconstruction, working memory and memory (Berr et al., 1998). Unfortunately the authors do not say which subtests differ. One study (Rujescu et al., 2003) reports higher intelligence scores in healthy middle-aged (mean age 44.6 years) Val-carriers than in Met-carriers in the HAWIE-R battery (Härting et al., 2000). The subtest with the strongest difference was the Digit

Symbol test, which assesses the speed of translating numbers into symbols, but the difference was also found in the Block Design task and the Similarities task (which assesses abstract thinking). All of these three significant differences showed the same gene dose effect (the more Val-alleles, the higher performance). The sample was torn randomly from the German population of Munich. However, these studies yield no explanation for the difference of the Val/Met group in our experiment. Because of the protein expression not only in the hippocampus, but also in wide parts of the brain, more pronouncedly in subcortical and very plastic structures (Bendheim et al., 1992), we do not suppose that episodic memory is the only function where differences could be observed. For example, there could also be differences in emotional functions or operant conditioning.

The second important finding of our study were anatomical differences between genotype groups, which we had proposed in hypothesis 5. Whole brain grey matter or white matter volumes did not differ significantly (Table 6), in contrast to a study which showed smaller white matter volumes in Met/Met carriers in a mixture of healthy and schizophrenic subjects (Rujescu et al., 2002). There was a non-significant tendency of smaller brains in carriers of the Val-allele compared to Met-carriers. However in voxel-based morphometry, Val-carriers showed regions with higher grey matter density compared to Met-carriers, while Met-carriers showed practically no regions with higher grey matter densities than Val-carriers. The reported results are corrected for brain size, but ANOVAs without correcting brain size yield qualitatively similar results (data not shown). These differences seem to be independent of the functional MRI results, since voxel-wise ANCOVAs of the functional contrasts which controlled for grey matter density in the same voxel did not change the pattern of overactivation of Val-carriers relative to Met-carriers. The anatomical finding in itself is interesting, because it gives us a second, independent

hint that the Met129Val polymorphism really influences enzymatic activity of the protein. It is not clear from our findings when this influence exerts. Prion protein is widely expressed during brain maturation and shows a shift from axons to synapses (Rieger et al., 1997). This points to a shift in function, from cellular plasticity to synaptic plasticity. Since voxel-based morphometry measures probably not only cell density, but also synaptic density, our grey matter concentration difference could map both kinds of plasticity, leading to a developmental difference in cell density or a post-birth difference in synaptic density due to activation-dependent synapse growth. It has also been shown that neurite outgrowth or retraction can be triggered over prion protein concentration (Rieger et al., 1997; Graner et al., 2000). Lack of prion protein does not abolish brain development, but can lead to anatomical abnormalities as in the hippocampal gyrus dentatus or CA3 (Colling et al., 1997).
The prion protein seems also to be involved in the regulation of the sleep cycles (Tobler et al., 1996). For our study, we asked subjects also for habitual sleep length and sleep length in the 'consolidation night' between learning of the first word list and recognition and did not find any significant differences or correlations with memory performance (however there was a tendency for longer sleep in the consolidation night in Met-carriers).

In summary, we find that Val-carriers have more difficulty to reach the same episodic memory performance than Met-carriers. This effect sums up with each Val-allele. This effect seems to be specific for episodic memory, because we do not see it in a working memory task. The difficulty of Val-carriers as compared to Met-carriers seems to start at a time point between 5 minutes and 30 minutes after learning and stays at least until 24 hours after learning. Additionally, we find higher grey matter concentrations in Val-carriers than in Met-carriers dispersed over wide parts of the

brain. These two effects, the difference in brain activation and the difference in grey matter density seem to be independent of each other. There are several candidate mechanisms that could explain the observed differences: Early LTP, late LTP, and possibly developmental differences in brain structure.

Since the Val-allele is the novel allele, it seems counterintuitive that it has just developed to confer difficulty in episodic memory. It seems also unlikely that the rare cases of Creutzfeldt-Jakob disease, against which Val/Met heterozygosity is a relative protection, have exerted enough evolutionary pressure to promote the Val-allele. More likely explanations are that the Val-allele has developed because it better fits into the changed genetic environment created by other genetic changes, or it confers advantages in trade-off of the memory-disadvantage. A hint for this is the higher intelligence in Val-carriers in the study of Rujescu et al. (2003). In our sample, this difference could not be found (although we partly administered the same neuropsychological tests), possibly because our sample was variance-constrained (2/3 students, and newspaper-recruited volunteers, who are often more intelligent than the mean population), an effect that probably acted stronger on intelligence than on memory. Although intelligence and memory often positively correlate, one can speculate that up to a certain degree one ability could also be compensated by the other, which could explain a possible memory-intelligence trade-off. In that sense, it could be a good choice to be a Val/Met heterozygous.

Extensive work has to be done on several levels to proceed in the question of how the prion protein and its Met129Val polymorphism influence cognitive function: From the systems side, we should try to replicate the memory and intelligence findings and find the processes where Val-carriers and Met-carriers differ most. From the cellular and molecular side, we should find out if and which enzymatic activities differ between metPrP and valPrP and if there are different patterns or levels of expression

in the cells. Also developmental studies comparing knock-out-knock-in mice and cell cultures with human metPrP versus valPrP would be of great help. There are already many studies with *PRNP* knockout mice or cells which found many functions of the protein (in this paper we reviewed only functions important for memory), but it is unclear what properties of the molecule are crucial to exert these functions. The Met129Val polymorphism could be of help to further investigate the mechanisms behind these functions, because it seems also to influence higher cognitive functions in humans, so it is likely that it influences also the cellular processes observed in knockout mice or cells. If the prion protein has a signal function which works over conformational switching, which one could suspect if comparing it to aplysia CPEB (see introduction or Si et al., 2003a, b), then it seems likely that this mechanism is altered by the Met129Val polymorphism, which influences the 3-dimensional structure of the protein. But even without a role of the switching mechanism in normal cells, there could be differences in enzymatic activities, which could influence the protein's likely functions in neuronal plasticity.

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Table 1. Prion Protein Genotype Groups

PRNP M129V	Met/Met (n=12)	Val/Met (n=12)	Val/Val (n=12)	χ2 or ANOVA p
Sex (female)	7	7	7	1
Handedness (right)	10	10	11	0.55
Age (M (SEM))	22.8 (0.3)	23.8 (0.6)	23.4 (0.8)	0.46
Years of education (M (SEM))	15.5 (0.3)	16.3 (0.4)	15.6 (0.5)	0.32
Genotypes APOE (3/3, 3/4, 2/3, 2/4)	9, 2, 0, 1	8, 1, 1, 2	10, 1, 0, 1	0.78
Genotypes 5HT2A (CC, CT)	8,4	11,1	8,4	0.26
Genotypes Kibra (CC)	3	5	5	0.62
correct answers “remember” 24h (M (SEM))	40.3 (1.6)	40.6 (1.7)	41.1 (1.5)	0.65

Table 2. Neuropsychological Data of the three Prion Genotype Groups (M, SEM)

PRNP Genotype	Met/Met (n=12)	Val/Met (n=12)	Val/Val (n=12)	ANOVA p
Memory				
Verbal Learning VLMT, Run 1	8.8 (0.7)	8.8 (0.8)	9.3 (0.8)	0.87
Verbal Learning VLMT, Run 5	14.5 (0.2)	14.4 (0.3)	14.5 (0.2)	0.96
Verbal Learning VLMT, Interference List	8.0 (0.6)	8.9 (0.7)	8.3 (0.6)	0.59
Verbal Learning VLMT, Recall after Interference	14.5 (0.3)	14.1 (0.4)	14.3 (0.3)	0.65
Verbal Learning VLMT, Late Recall	14.8 (0.1)	13.7 (0.6)	14.3 (0.4)	0.22
Verbal Learning VLMT, Recognition	15.0 (0.0)§	14.7 (0.2)	14.9 (0.1)	0.13
Verbal Association Learning WMS-R, Runs 1-3	21.9 (0.6)	21.3 (0.9)	21.8 (0.5)	0.76
Verbal Association Learning WMS-R, Late Recall	7.8 (0.1)	7.7 (0.2)	7.8 (0.1)	0.73
Visual Association Learning WMS-R, Runs 1-3	17.0 (0.4)	16.8 (0.5)	16.8 (0.4)	0.90
Visual Association Learning WMS-R, Late Recall	6.0 (0.0)§	6.0 (0.0)§	5.8 (.17)	0.38
Intelligence				
Digit Span (HAWIE-R)	15.6 (1.3)	16.0 (1.0)	14.8 (0.8)	0.69
Mosaic Test (HAWIE-R)	43.2 (1.5)	40.1 (2.0)	44.8 (1.9)	0.33
Abstract Thinking (HAWIE-R)	27.8 (0.6)	27.3 (0.8)	26.4 (1.2)	0.56
Vocabulary (MWT-B)	30.7 (0.8)	31.4 (0.8)	30.3 (0.9)	0.64
Executive Functions				
Interference (Stroop: Time Run 3)	20.7 (1.6)	20.9 (1.8)	18.9 (1.3)	0.63

§ all subjects in these groups reached the maximum

Table 3. Performance in fMRI tasks (M (SEM))

PRNP Genotype	Met/Met (n=12)	Val/Met (n=12)	Val/Val (n=12)	ANOVA p
Free Recall				
Day 1, Correct	30.5 (1.7)	32.4 (1.7)	27.8 (1.0)	0.11
Day 2, Correct	20.3 (1.9)	23.6 (1.6)	21.5 (1.6)	0.39
30min Recognition				
Hits 'Remember'	40.9 (2.2)	39.6 (1.7)	39.8 (2.0)	0.88
Hits 'Know'	5.8 (1.4)	7.0 (1.1)	5.4 (0.9)	0.6
Miss	3.3 (1.1)	3.4 (0.9)	4.7 (1.3)	0.65
Mean Reaction Times Hits 'Remember' (ms)	2087 (131)	2187 (157)	2307 (137)	0.55
24h Recognition				
Hits 'Remember'	40.3 (1.6)	40.6 (1.7)	41.1 (1.5)	0.93
Hits 'Know'	7.3 (1.2)	6.5 (1.2)	6.0 (1.0)	0.71
Miss	2.4 (0.6)	2.9 (1.1)	2.9 (0.8)	0.89
Mean Reaction Times Hits 'Remember' (ms)	1982 (131)	2218 (174)	2400 (194)	0.22
Distracters				
False Alarms 'Remember'	4.2 (1.0)	4.5 (1.8)	3.6 (0.9)	0.58
False Alarms 'Know'	18.6 (3.4)	15.5 (3.9)	14.1 (2.3)	0.61
Correct Rejections	77.0 (4.0)	78.8 (4.5)	82.2 (2.3)	0.62
Mean Reaction Times Correct Rejections (ms)	2918 (206)	3320 (265)	3069 (122)	0.39
Baseline Task				
Identical', Correct	12.0 (0.0) #	12.0 (0.0) #	12.0 (0.0) #	
Identical', Mean Reaction Times (ms)	1584 (112)	1568 (98)	1686 (137)	0.74
Same', Correct	12.0 (0.0) #	12.0 (0.0) #	11.9 (0.3)	
Same', Mean Reaction Times (ms)	1647 (118)	1796 (139)	1913 (147)	0.39
Different', Correct	26.0 (0.0) #	25.8 (0.5)	25.9 (0.3)	

Different', Mean Reaction Times (ms)	1700 (134)	1796 (153)	1828 (138)	0.8
Working Memory Task				
X-Target', Hits - False Alarms	12.8 (0.1)	12.9 (0.1)	12.9 (0.1)	0.77
X-Target', Mean Reaction Times Hits (ms)	505 (22)	536 (27)	537 (20)	0.55
2-Back', Hits - False Alarms	9.1 (0.9)	8.7 (1.2)	9.8 (0.9)	0.74
2-Back', Mean Reaction Times Hits (ms)	673 (35)	706 (35)	732 (38)	0.52

All Subjects Reached the Maximum

Table 4. Brain Activations (Whole Sample, n=36) in Different Contrasts (1-sample t-test; p<.05, Family Wise Error Corrected, extent threshold 12 voxels, for hippocampus 5 voxels)

			MNI Coordinates				
Brain Area	Side	BA	x	y	z	Z	# vox
Rem 30min > False Alarms							
Middle Frontal Gyrus	R	8	30	24	46	5.45	26
	R	8	28	34	52	5.35	26
Middle Temporal Gyrus	L	21	-60	-10	-20	5.40	27
	R	37	62	-56	-6	5.97	70
Superior Temporal Gyrus	L	22	-62	-48	16	5.28	12
Parahippocampal Gyrus	R	28	24	-18	-22	5.74	88
Angular Gyrus	L	39	-48	-72	38	6.04	344
Inferior Parietal Lobule	R	39	48	-70	24	6.54	598
	L	40	-56	-46	50	5.61	17
Posterior Cingulate	L	31	-10	-54	36	6.67	1354
False Alarms > Rem 30min							
no significant activations							
Rem 24h > False Alarms							
Middle Frontal Gyrus	R	46	50	40	0	5.22	21
	R	8	22	40	50	5.26	21
	R	8	28	22	44	5.25	28
Claustrum	R		34	-10	-2	5.92	144
Inferior Temporal Gyrus	L	20	-58	-6	-24	5.82	42
	R	20	54	-8	-30	5.57	30
Middle Temporal Gyrus	R	21	58	-30	2	5.41	23
Superior Temporal Gyrus	L	22	-60	-50	16	5.47	90
	R	42	58	-26	14	5.35	40

Parahippocampal Gyrus	L	36	-22	-6	-32	5.67	59
	L	36	-28	-18	-30	5.48	33
	R	28	24	-20	-24	5.85	119
Inferior Parietal Lobule	L	40	-62	-42	30	5.25	29
	R	39	48	-70	24	6.69	1245
	R	40	60	-42	48	5.41	15
Middle Occipital Gyrus	L	19	-44	-80	32	6.69	546
False Alarms > Rem 24h							
Inferior Frontal Gyrus	R	11	32	26	-4	5.76	42
2-Back > X-Target							
Inferior Frontal Gyrus	L	47	-34	24	-6	7.69	623
	R	47	38	24	-8	>7.7	7502
Middle Frontal Gyrus	L	10	-32	56	6	5.77	213
Fusiform Gyrus	L	19	-48	-66	-14	5.25	32
Superior Parietal Lobule	Both	7	14	-70	56	7.66	6145
X-Target > 2-Back							
Inferior Frontal Gyrus	L	11	-34	36	-16	5.88	34
Insula	L		-40	-14	16	6.88	1241
	R		44	-22	18	7.28	1189
Parahippocampal Gyrus	L	36	-26	-16	-24	6.25	262
	R	34	30	2	-24	6.25	101
	R	35	28	-36	-16	5.67	35
	R	28	26	-18	-24	5.59	21
Postcentral Gyrus	L	3	-42	-28	60	5.45	33
	R	1	24	-30	66	5.18	21
Precentral Gyrus	L	4	-48	-12	32	5.54	12
	R	4	40	-26	68	5.65	24

Table 5. Activation Differences between Prion Gene Groups: (Met/Met vs Val/Met vs Val/Val, 12:12:12) in Different Contrasts (ANOVAs and consecutive 2-sample T-Tests; p<.001 uncorrected, extent threshold 12 voxels, in hippocampus 5 voxels)

			MNI coordinates				
Brain Area	Side	BA	x	y	z	Z	# vox
Rem 30min > False Alarms							
Met/Met > Val/Val							
no significant differences							
Val/Val > Met/Met							
Inferior Frontal Gyrus	L	45	-40	36	6	3.83	69
	R	44	40	6	24	3.84	56
	R	47	44	26	-8	3.73	55
Middle Frontal Gyrus	L	9	-38	24	26	3.79	23
	L	9	-28	42	32	3.50	13
Thalamus	R		10	-12	6	3.65	31
Caudate	L		-10	10	-4	4.11	45
Fusiform Gyrus	L	19	-26	-92	-16	3.88	34
	R	20	30	-34	-28	4.02	62
Middle Temporal Gyrus	L	21	-64	-46	4	4.14	64
	L	21	-52	-2	-24	3.64	28
	L	21	-50	-50	0	3.41	29
	R	21	66	-34	4	4.46	28
	R	21	60	-10	-10	3.83	29
Insula	L		-28	10	12	4.28	21
Parahippocampal Gyrus	L	28	-22	-14	-30	4.04	58
Superior Parietal Lobule	L	7	-26	-72	32	4.32	115
	L	5	-10	-44	58	3.55	13
	R	7	18	-68	34	3.47	12

Angular Gyrus	L	39	-48	-76	24	4.21	35
Inferior Parietal Lobule	R	40	36	-24	24	3.69	18
Posterior Cingulate	R	23	10	-58	14	3.48	26
Met/Met > Val/Met							
no significant differences							
Val/Met > Met/Met							
Inferior Frontal Gyrus	R	44	50	16	16	3.59	12
Middle Frontal Gyrus	L	46	-34	42	14	3.47	14
Claustrum	R		28	6	-10	3.56	15
Superior Temporal Gyrus	L	22	-62	-44	6	3.43	49
Parahippocampal Gyrus	L	27	-22	-22	-16	3.80	23
Val/Met > Val/Val							
no significant differences							
Val/Val > Val/Met							
Precuneus	R	7	20	-68	32	4.55	23
Inferior Parietal Lobule	R	39	36	-72	26	3.86	24
Superior Occipital Gyrus	L	19	-28	-84	28	3.62	12

Rem 24h > False Alarms

Met/Met > Val/Val							
no significant differences							
Val/Val > Met/Met							
Inferior Frontal Gyrus	L	44	-58	14	22	3.76	51
Middle Temporal Gyrus	L	21	-52	2	-24	3.82	30
Insula	R		32	10	-8	3.71	26
Superior Parietal Lobule	L	7	-28	-74	32	3.46	24
Posterior Cingulate	L	23	-6	-22	32	3.53	19
Met/Met > Val/Met							

no significant differences							
Val/Met > Met/Met							
Inferior Frontal Gyrus	R	45	48	20	14	3.45	18
	L	44	-58	14	20	3.55	21
Middle Frontal Gyrus	L	46	-36	44	12	3.53	19
Putamen	R		28	8	-8	4.01	48
Middle Temporal Gyrus	L	21	-56	-54	-4	3.71	14
Parahippocampal Gyrus	L	28	-20	-14	-30	3.51	15
Hippocampus (Body)	L		-34	-26	-14	3.37	8
Val/Met > Val/Val							
no significant differences							
Val/Val > Val/Met							
Middle Temporal Gyrus	R	21	46	-50	0	3.69	17

2-Back > X-Target

Met/Met > Val/Val							
no significant differences							
Val/Val > Met/Met							
no significant differences							
Met/Met > Val/Met							
no significant differences							
Val/Met > Met/Met							
Inferior Temporal Gyrus	R	20	40	0	-28	3.53	13
Superior Temporal Gyrus	L	41	-56	-18	6	3.37	17
Val/Met > Val/Val							
Inferior Frontal Gyrus	R	11	24	28	0	3.65	15
Val/Val > Val/Met							
Inferior Frontal Gyrus	R	44	54	14	4	3.32	7

Table 6. Whole Brain Volumes of the Prion Genotype Groups (M (SEM); ccm3)

PRNP Genotype	Met/Met (n=12)	Val/Met (n=12)	Val/Val (n=12)	ANOVA p
Grey Matter	697 (21)	690 (18)	663 (16)	0.39
White Matter	431 (17)	417 (12)	398 (10)	0.22
Cerebrospinal Fluid	462 (18)	449 (11)	432 (13)	0.34
Total (Grey, White and CSF)	1589 (54)	1558 (38)	1492 (38)	0.30

Figure 1.

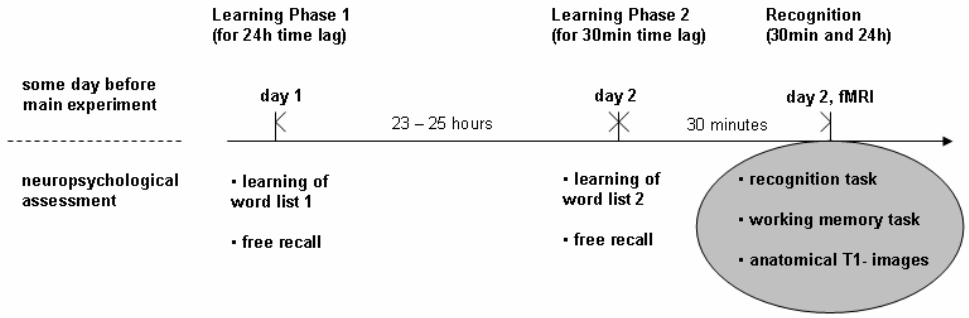
Learning Phase 1
(for 24h time lag)
Learning Phase 2
(for 30min time lag)
Recognition
(30min and 24h)
some day before
main experiment
day 1
23 – 25 hours
day 2
30 minutes
day 2, fMRI
neuropsychological
assessment
▪ learning of
word list 1
▪ free recall
▪ learning of
word list 2
▪ free recall
▪ recognition task
▪ working memory task
▪ anatomical T1- images

Figure 2.

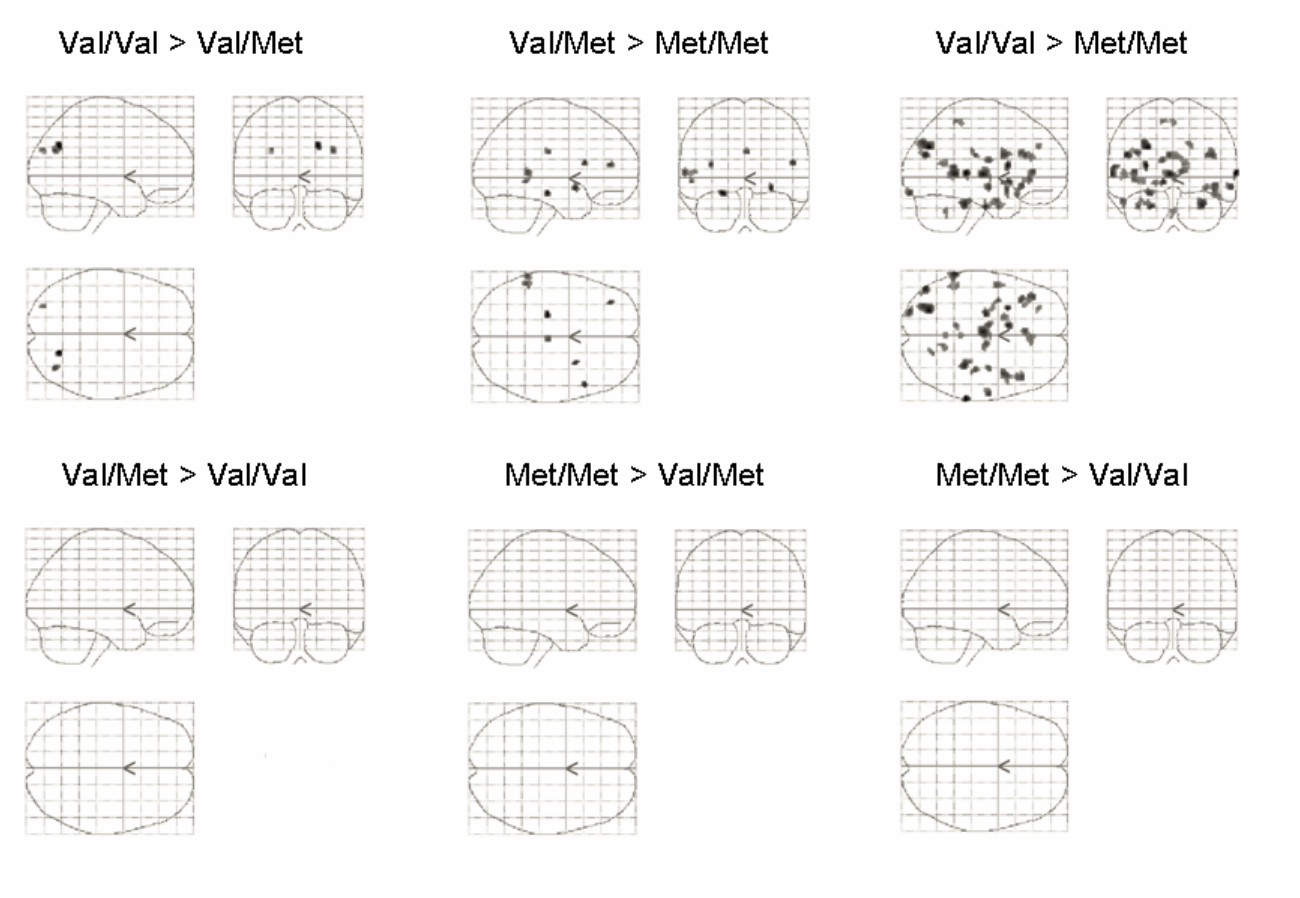
Val/Val > Val/Met
Val/Met > Met/Met
Val/Val > Met/Met
Val/Met > Val/Val
Met/Met > Val/Met
Met/Met > Val/Val

Figure 3.

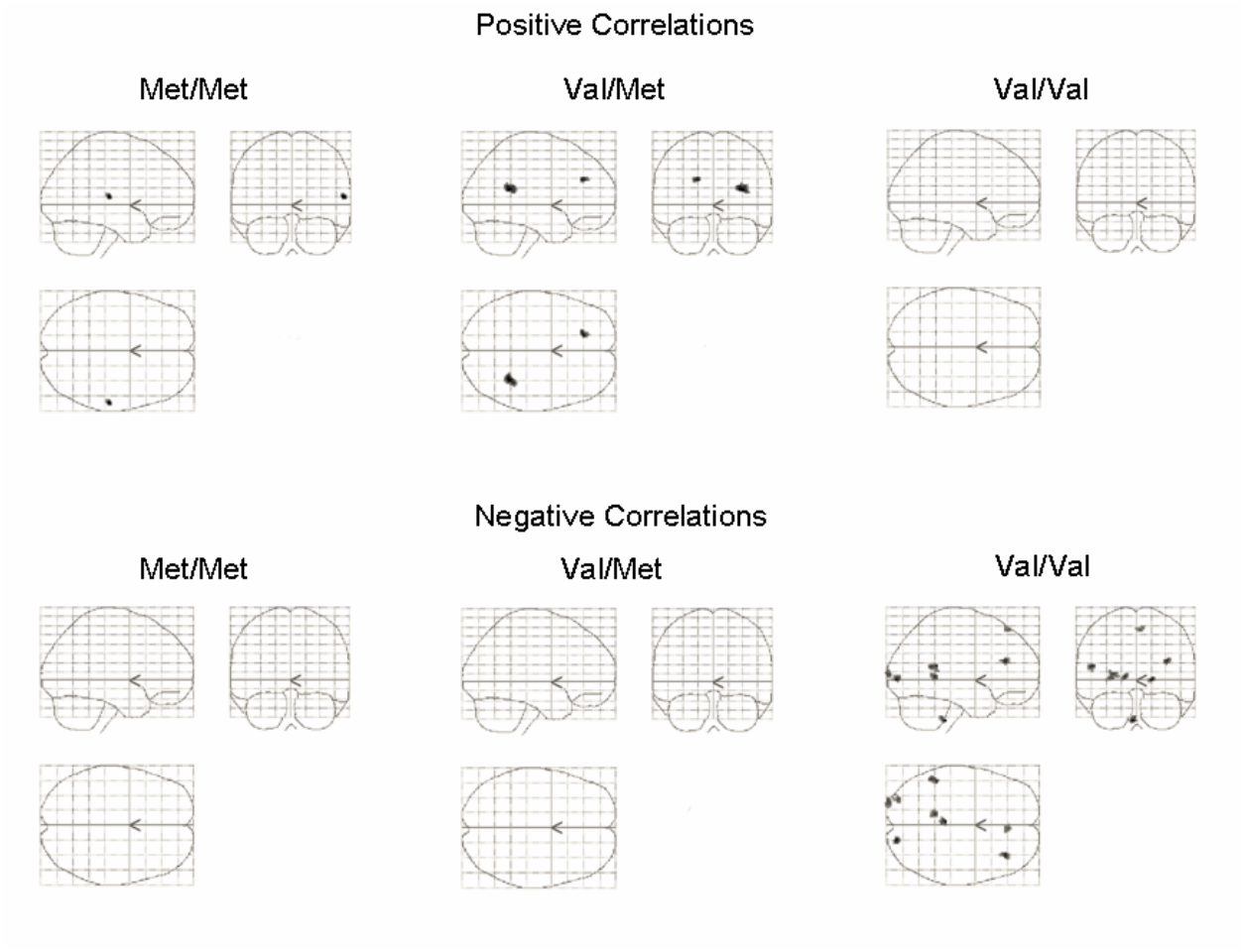
Positive Correlations
Met/Met
Val/Met
Val/Val
Negative Correlations
Met/Met
Val/Met
Val/Val

Figure 4.

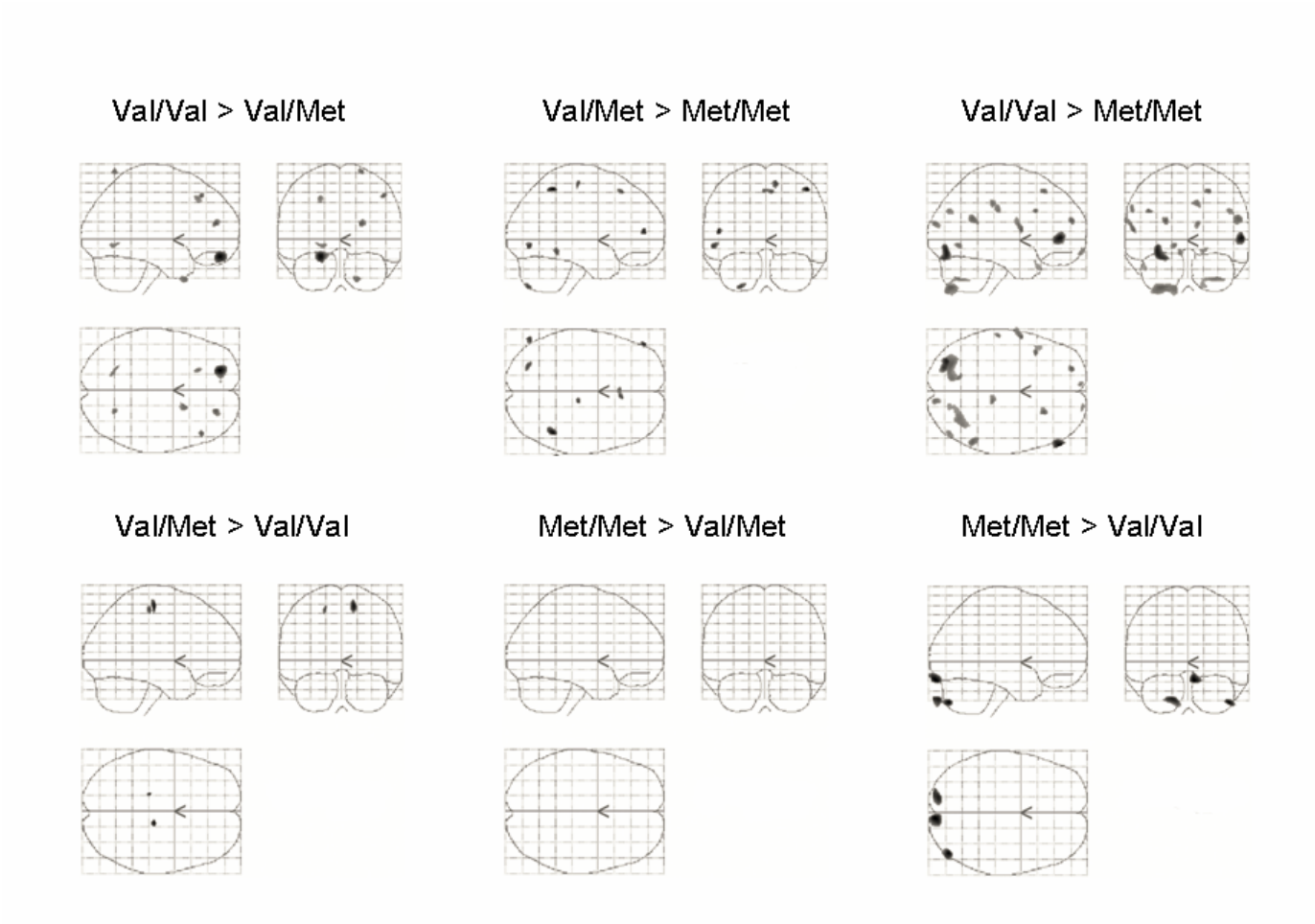
Val/Val > Val/Met
Val/Met > Met/Met
Val/Val > Met/Met
Val/Met > Val/Val
Met/Met > Val/Met
Met/Met > Val/Val

Figure 5.

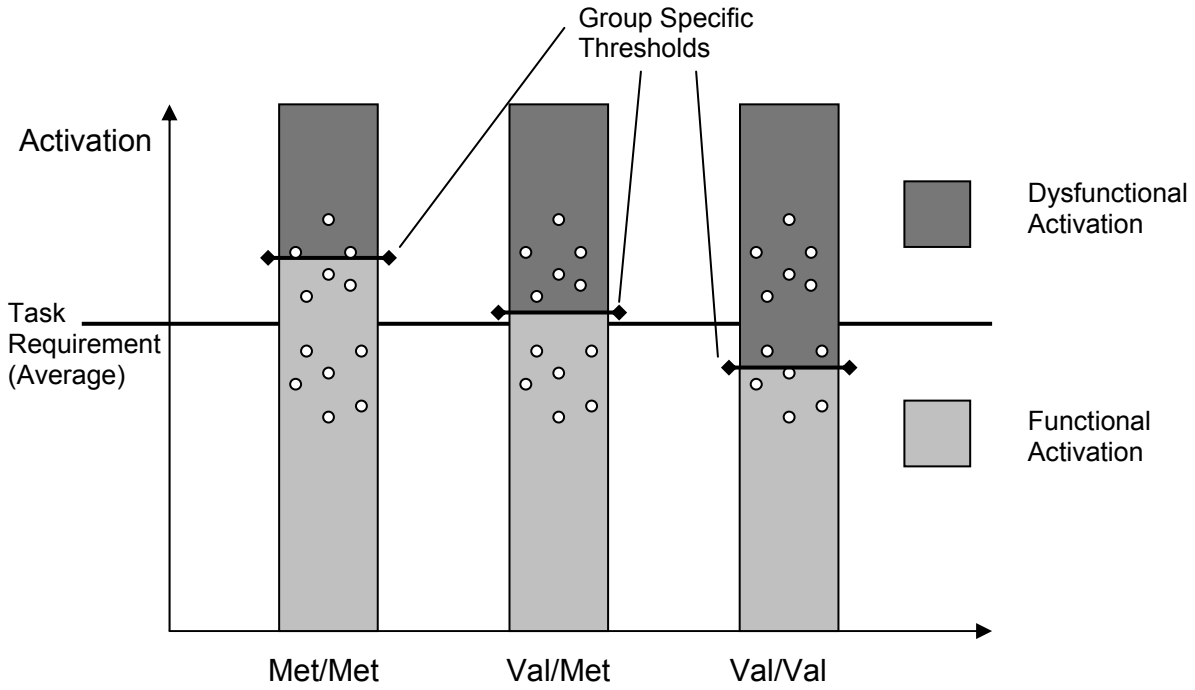
Group Specific
Thresholds
Activation
Task
Requirement
(Average)
Dysfunctional
Activation
Functional
Activation
Met/Met
Val/Met
Val/Val

Figure legends

Figure 1. Experimental procedure

Figure 2. Comparison of activations in the contrast ‘30min Recognition minus False Alarms’ between prion genotype groups (Anovas with subsequent paired comparisons; only ‘Remember’-answers; n=12 per group). The same ‘transitive’ pattern (Val/Val > Val/Met > Met/Met) can also be seen in the 24h condition. Clusters were plotted for p>.001 (uncorrected) and an extent threshold of 12 voxels or more (exception hippocampus: 5 voxels or more).

Figure 3. Comparison of correlations of the activations in the contrast ‘30min Recognition minus False Alarms’ with recognition performance (hits minus false alarms; only ‘Remember’-answers; n=12 per group). The same pattern (Met/Met and Val/Met only positive correlations, Val/Val only negative correlations) can also be seen in the 24h condition. Clusters were plotted for p>.001 (uncorrected) and an extent threshold of 12 voxels or more (exception hippocampus: 5 voxels or more).

Figure 4. Voxel-wise comparison of local grey matter concentrations between prion genotype groups (Ancovas with whole brain volume as a covariate and subsequent pair-wise comparisons; n=12 per group). Clusters were plotted for p>.001 (uncorrected) and an extent threshold of 100 voxels or more (exception hippocampus: 40 voxels or more).The settings were chosen to match the functional activity maps as closely as possible (voxels in the anatomical images were 1x1x1 mm, in the functional data 2x2x2 mm; therefore the 8-times larger number of voxels for the extent threshold).

Figure 5. Model to explain the fMRI results: The model assumes that activation up to a certain degree (the group-specific threshold) is functional and increases performance, while it decreases performance if lying above the group-specific threshold. Since carriers of more Val-alleles seem to have more difficulty with memory, their threshold lies lower than the threshold of carriers of less Val-alleles. Therefore most Val-carriers at the same performance level (which was matched) tend to enter the dark grey activation level, where their performance does not profit from additional activation (negative correlations within genotype group), whereas Met-carriers would still profit from additional activation (positive correlations within group).

Supplementary Tables:

Table S1. fMRI Activations in the Word Recognition Task, Compared to Low-Level Baseline (Compare the First and Last Letter of a Letter String; Whole Sample, n=36) in Different Contrasts (1-Sample T-Test; p<.05, Family Wise Error Corrected, Extent Threshold 12 Voxels, in Hippocampus 5 Voxels)

			MNI Coordinates				
Brain Area	Side	BA	x	y	z	Z	# vox
Rem 30min > Compare Letters							
Inferior Frontal Gyrus	L	47	-48	22	-12	7.52	1321
	L	8	-42	12	42	5.58	87
	R	47	36	22	-14	5.75	135
Middle Frontal Gyrus	L	10	-32	48	4	6.20	157
	L	6	-42	8	56	5.72	65
Superior Frontal Gyrus	L	9	-12	56	34	5.54	52
	L	10	-18	60	24	5.26	13
Caudate	L		-12	6	12	5.98	108
	R		10	10	6	7.68	92
Middle Temporal Gyrus	L	21	-62	-40	-6	6.53	331
	L	21	-56	-10	-18	6.08	58
Supramarginal Gyrus	L	40	-40	-64	42	6.73	621
Posterior Cingulate	L	23	-6	-26	26	5.59	26
Compare Letters > Rem 30min							
Inferior Frontal Gyrus	R	44	52	8	30	5.49	96
Middle Frontal Gyrus	R	46	50	42	0	6.39	91
Medial Frontal Gyrus	L	6	-22	-14	54	6.30	92
	R	6	28	6	68	5.02	178
Thalamus	L		-14	-24	4	7.09	52
	L		-20	-32	10	5.51	16

	R		14	-24	-4	5.65	52
Inferior Temporal Gyrus	L	20	-40	-4	-22	5.77	19
	R	20	40	2	-28	5.92	53
Insula	L		-42	2	4	6.06	283
	R		36	8	6	6.01	65
Postcentral Gyrus	L	1	-50	-28	46	6.80	1566
Rem 24h > Compare Letters							
Inferior Frontal Gyrus	L	47	-48	22	-12	>7.5	1745
	R	47	36	22	-14	7.24	265
Middle Frontal Gyrus	L	8	-40	10	42	5.89	263
	L	6	-40	2	56	5.53	42
Superior Frontal Gyrus	L	9	-14	54	30	5.82	291
Caudate	L		-12	8	10	6.68	201
	R		10	14	6	5.77	154
Middle Temporal Gyrus	L	21	-60	-40	-6	6.55	392
	L	21	-56	-10	-18	5.70	70
Angular Gyrus	L	39	-48	-72	40	6.24	434
Compare Letters > Rem 24h							
Inferior Frontal Gyrus	R	45	48	38	4	5.72	33
	R	44	52	8	32	5.67	117
Middle Frontal Gyrus	R	6	30	-4	56	5.75	113
Thalamus	L		-20	-30	2	2.68	39
	R		18	-30	4	5.21	16
Inferior Temporal Gyrus	R	20	40	2	-26	6.12	36
Middle Temporal Gyrus	L	21	-40	-4	-22	5.29	13
Superior Temporal Gyrus	L	22	-56	-2	0	6.01	26
Insula	L		-42	2	6	6.27	95

	L		-40	-16	-8	5.38	27
	R		34	8	4	5.71	32
Superior Parietal Lobule	R	7	30	-62	42	7.51	5961
Postcentral Gyrus	L	2	-48	-36	60	6.72	1222
Posterior Cingulate	L	31	-14	-30	42	6.12	60
Inferior Occipital Gyrus	L	19	-42	-74	-14	7.41	1813
False Alarms > Compare Letters							
Inferior Frontal Gyrus	L	47	-28	22	-10	7.23	943
	L	47	-42	42	-8	5.65	60
	R	47	38	22	-12	7.12	358
Compare Letters > False Alarms							
Inferior Frontal Gyrus	R	44	52	6	30	5.90	58
Middle Frontal Gyrus	R	46	50	40	0	6.27	107
	R	6	28	14	52	5.66	106
Thalamus	L		-16	-30	0	5.81	144
	R		18	-28	4	5.83	147
Middle Temporal Gyrus	R	21	58	-2	-18	5.56	26
	L	37	-60	-62	-8	>7.5	15625
Superior Temporal Gyrus	R	22	60	2	-4	5.53	82
Fusiform Gyrus	L	37	-40	-56	-4	5.30	16
	R	20	26	-16	-26	5.24	16
Claustrum	R		36	-10	-4	7.49	1055
Parahippocampal Gyrus	L	36	-22	-4	-30	5.44	27
Postcentral Gyrus	L	7	-24	-38	58	5.42	15
Inferior Parietal Lobule	L	40	-64	-30	24	7.00	1612
Inferior Occipital Gyrus	R	18	36	-86	-10	5.52	35

Table S2. Significant Correlations of Activation Contrasts with Behavioral Measures (in parentheses) in Prion Gene Groups: (Met/Met, Val/Met, Val/Val, 12:12:12) for Different Contrasts (p<.001 Uncorrected, Extent Threshold 12 Voxels)

			MNI Coordinates				
Brain Area	Side	BA	x	y	z	Z	# vox
Rem 30min > False Alarms with (Hits 30min - False Alarms)							
Met/Met, r>0							
Superior Temporal Gyrus	R	22	58	-24	10	3.69	12
Met/Met, r<0							
no significant correlations							
Val/Met r>0							
Medial Frontal Gyrus	L	8	-18	36	28	4.10	27
Val/Met r<0							
no significant correlations							
Val/Val, r>0							
no significant correlations							
Val/Val r<0							
Middle Frontal Gyrus	R	46	34	34	22	3.95	26
Superior Temporal Gyrus	L	22	-50	-44	16	3.88	32
Posterior Cingulate	L	30	-12	-46	4	3.82	23
Middle Occipital Gyrus	L	18	-26	-98	6	3.65	18
	L	18	-28	-88	2	3.63	18
Cuneus	R	17	18	-90	0	4.13	18
Rem 24h > False Alarms with D_24 Rem							
Met/Met, r>0							
no significant correlations							

Met/Met, r<0

no significant correlations

Val/Met r>0

Anterior Cingulate	R	24	10	-16	46	3.81	22
Inferior Temporal Gyrus	R	37	50	-54	-22	4.04	14
Superior Temporal Gyrus	R	22	52	-48	14	4.07	102
Fusiform Gyrus	R	19	44	-68	-8	3.89	26

Val/Met r<0

no significant correlations

Val/Val, r>0

Caudate	L		-12	24	12	3.77	12

Val/Val r<0

Inferior Frontal Gyrus	L	44	-42	10	22	4.57	42
	L	47	-40	40	-6	3.96	34
	L	47	-34	24	0	3.71	31
	L	47	-22	34	-4	3.71	38
	L	45	-56	20	28	3.51	53
	R	47	38	40	-8	4.26	24
	R	9	48	12	34	3.42	15
Middle Frontal Gyrus	L	46	-38	42	22	4.72	80
	L	6	-26	-2	32	3.98	26
	R	9	36	52	22	3.86	102
Putamen	R		18	16	-2	3.50	18
Superior Temporal Gyrus	R	38	54	16	-14	3.82	33
Insula	L		-34	10	-10	3.70	22
Superior Parietal Lobule	R	7	10	-74	54	3.91	25
Postcentral Gyrus	L	1	-60	-22	38	4.11	43
	L		-38	-28	52	4.10	87

Inferior Occipital Gyrus	R	18	34	-88	-10	3.97	44
Cuneus	L	19	-8	88	42	4.50	83

2-Back > X-Target with (Hits - False Alarms) 2-Back

Met/Met, r>0							
Insula	R		44	-8	0	3.90	33
Met/Met, r<0							
Inferior Parietal Lobule	L	7	-38	-62	48	3.70	41
	R	40	42	-58	52	4.05	30
Val/Met r>0							
Middle Frontal Gyrus	L	10	-28	48	2	3.57	18
	R	6	24	12	66	3.53	15
Medial Frontal Gyrus	R	6	16	-26	60	4.75	137
Inferior Temporal Gyrus	L	37	-48	-62	-18	3.33	13
	R	37	52	-58	-16	4.77	185
Middle Temporal Gyrus	R	21	62	-6	-4	3.48	16
Superior Temporal Gyrus	R	22	46	-44	14	3.59	13
Fusiform Gyrus	R	37	32	-50	-14	3.92	16
Paracentral Lobule	R	5	6	-44	64	3.77	26
Postcentral Gyrus	L	1	-28	-42	44	4.07	51
	L	1	-18	-42	74	4.03	37
Inferior Parietal Lobule	L	40	-52	-50	20	3.87	36
Superior Parietal Lobule	L	5	-28	-50	64	3.44	21
	R	7	18	-80	34	4.41	151
Inferior Occipital Gyrus	R	19	40	-78	-10	3.56	16
Middle Occipital Gyrus	L	19	-46	-70	-12	3.46	18
	R	19	40	-76	10	3.96	76
Superior Occipital Gyrus	L	19	-26	-86	38	4.08	19

	L	19	-18	-86	18	3.94	19
Lingual Gyrus	R	19	22	-52	-6	3.89	26
Val/Met r<0							
no significant differences							
Val/Val, r>0							
Superior Frontal Gyrus	L	8	-16	26	38	3.66	16
	L	8	-6	26	46	3.57	33
Caudate	R		8	16	0	4.23	18
Inferior Parietal Lobule	R	40	58	-56	40	3.85	14
Superior Parietal Lobule	R	7	14	-52	36	3.78	13
Middle Occipital Gyrus	R	19	48	-80	12	3.80	14
Val/Val r<0							
Amygdala	R		20	-8	-12	3.98	13
Inferior Parietal Lobule	R	40	50	-30	58	3.78	33

Table S3. Grey Matter Concentration Differences between Prion Gene Groups: (Met/Met vs Val/Met vs Val/Val, 12:12:12 ANCOVAs with Whole Brain Volume as a Covariate and consecutive 2-sample Comparisons; p<.001 uncorrected; Extent Threshold 100 Voxels, in Hippocampus 40 Voxels; Corresponding to Extent Threshold for fMRI Data)

			MNI Coordinates				
Brain Area	Side	BA	x	y	z	Z	# vox
Met/Met > Val/Val							
Lingual Gyrus	R	18	6	-93	-20	3.68	998
Cerebellum	L		-16	-91	-41	3.59	818
Cerebellum	R		45	-80	-44	3.66	465
Val/Val > Met/Met							
Orbital Gyrus	L	11	-7	65	-14	3.45	115
	R	10	16	68	-13	3.36	122
Middle Frontal Gyrus	L	9	-23	56	21	3.78	190
	L	9	-42	17	31	3.75	128
	R	8	23	26	53	3.77	223
Superior Temporal Gyrus	L	38	-45	20	-31	3.23	123
Precentral Gyrus	L	4	-59	1	10	3.8	329
Postcentral Gyrus	L	1	-60	-23	33	3.53	152
Inferior Parietal Lobule	R	40	56	-52	22	3.69	447
	R	39	45	-77	31	3.42	183
Posterior Cingulate	R	31	9	-30	40	3.6	257
Fusiform Gyrus	L	19	-33	-80	-14	4.57	1281
Lingual Gyrus	L	18	-14	-66	-7	3.56	155
Cuneus	R	18	10	-92	18	3.56	235
Cerebellum	L		-29	-75	-52	3.8	2154
	R		32	-66	-42	3.53	819

Met/Met > Val/Met

no significant differences

Val/Met > Met/Met

Middle Frontal Gyrus	L	10	-52	50	10	3.7	102
Medial Frontal Gyrus	R	8	7	26	52	3.4	205
	R	6	10	-22	60	3.43	122
Middle Temporal Gyrus	L	37	-57	-76	-7	3.49	196
Inferior Parietal Lobule	R	40	45	-50	54	3.67	262
Cerebellum	L		-27	-76	-50	3.42	193

Val/Met > Val/Val

Medial Frontal Gyrus	R	6	13	-22	59	3.64	281
Precentral Gyrus	L	4	-18	-28	55	3.45	103

Val/Val > Val/Met

Inferior Frontal Gyrus	L	11	-21	50	-19	4.55	1287
Middle Frontal Gyrus	L	8	-22	28	44	3.4	169
	R	9	46	30	47	3.67	124
Superior Frontal Gyrus	R	10	23	46	18	3.67	283
Fusiform Gyrus	L	19	-23	-63	-6	3.42	177

3 Discussion

In this broader discussion, I will try to give an overview of the methodological problems of such kind of studies (chapter 3.1) and an impression of the field of research and the expectations that I have for its future (chapter 3.2).

3.1 Methodological Considerations

3.1.1 Problems with Genetic Studies in General

One of the major problems of genetic studies of normal cognitive functions is *effect size*: Effects sizes are most of the times very small (it is already amazing if they reach the scale of 1%, if you think of the high number of variants and the often very indirect action of the genes). In consequence, to statistically prove such small effects, we need enormously *large samples*. Large samples give of course large power, because random effects are averaged out, but the danger to be caught by systematic bias rises: The effect size of a systematic bias is the same for a large sample as for a small sample, but it will rather get significant and be considered as a real effect in a large sample. The standard solution of this problem is to take two independent large samples. It seems very unlikely that in both samples the same effect gets statistically significant. Note that this is true for random effects, but not for systematic biases: Because they *are* systematic, they will show up not just in these two samples, but in every sample you would want to investigate. A better idea to get rid of systematic bias probably would be to choose different kinds of account and see if results converge. This is what we tried with our fMRI studies, attempting to control for behavioral effects and using different measures for memory performance, extracted from the fMRI signal. The fMRI signal is probably also one step nearer to the genes as the behaviour (it probably measures rather the underlying processes than just behavioral sum scores), which should increase the power of such an account. The problem arising there is one of interpretation: A difference in fMRI signal can mean many different things (partial volume effects, different degrees of anxiety of the scanning procedure, difference in coffee consumption and so on), so no one would conclude on genetic effects just from differences in the fMRI signal without behavioral

differences and/or further good reasons to assume that genetic differences could exist, like biochemical results of what gene products do in the context of the measured cognitive process.

Another problem is that most of the variants are *rare*. The single nucleotid polymorphisms reviewed in this work are with one exception more frequent than about 25%. Rarer variants are of course difficult to find (see our PS1 study, Mondadori et al., 2006b): You need an even larger population to have at least 10-12 carriers of a rare variant. For example, if there is a polymorphism with a frequency of 10%, the probability to be a homozygous carrier is just 1%, so you would have to survey 1000 volunteers to get 10 homozygous carriers. In the study with the prion protein Met129Val polymorphism, we were quite lucky in this respect to get in total about 16 Val/Val carriers (frequency of Val/Val is around 12%, Palmer et al., 1991) and to have still 12 carriers after drop-outs and performance matching (from around 350 volunteers genotyped that time, about half agreed to take part in fMRI studies). Of course the problem is also to get money if you wish to investigate a genetic variant that has a frequency of around 5%. This does not mean that investigating these rare variants would not be interesting: They may elucidate the influence of an altered protein on cognitive functions, which could maybe not be assessed otherwise, for example if the cognitive function can not be measured in animals.

A severe problem are *multiple comparisons*. In principle, if you wanted to investigate the effects of a genetic variant, you would have to match for the whole rest of the genome *and* all environmental variables. Matching genes can be done more or less with animals (same genetic background) but not in humans, of course. In humans, if you want to control one gene while investigating another, you will lose half of your sample size in the best case. Of course some statistical tools like logistic regression try to solve this problem, but they do it only partly, since their results depend for example on the sequence of variables you feed into the procedure. One attempt to control genetic backgrounds in humans is to check for population stratification with the use of unrelated single nucleotid polymorphisms (see chapter 1.1.4) dispersed over the whole genome. If their distribution does not differ between groups, stratification is controlled on the whole genome-level (Pritchard and Rosenberg, 1999). Of course this is a good idea but no guarantee that some other polymorphism may be in *linkage disequilibrium* to the investigated polymorphism and either impose their effect on the measured polymorphism, or obstruct a real effect (the

polymorphisms near to the investigated polymorphism are more likely to be correlated, since they are more likely to be inherited together; see the mixing process during meiosis, chapter 1.1.5). In our fMRI studies, we did the best we could, which is matching 3-4 other polymorphisms that have shown to influence memory. Up to a certain degree, this matches also nearby polymorphisms, because they are often inherited together. Of course this is not a big help since there could be many not yet discovered polymorphisms that could have been in linkage disequilibrium to our investigated polymorphisms.

Another problem which is more important for genetic research than for research in 'general psychology' (which deals with effects that are the same in all human beings) is *selection bias*. Our group did the best they could to look not only at a population of psychology- and medicine-students interested in memory experiments, but also a second population of persons reading a very common free newspaper (which was a necessary condition to see the ad). Of course this is already an exceptional part of the Swiss population, because interest in this kind of work is a sign of over-average intellectual abilities, which could be seen also in the professional categories and intelligence measures of this second population, which were as high as the intelligence measures of the students (except education-dependent measures like vocabulary tests or tests of general knowledge). This fact made it easier for us to do the experiments (average people would have had problems to get along with the instructions), but our results might underestimate the variability of memory in the general population. I suppose that this must not be a problem, because we measure the part of the variability conferred by a single polymorphism relative to the variability in our study population. Having homogeneous groups is possibly an additional control over genetic backgrounds and a part of other confounding variables. An interesting exception of a study which randomly selected inhabitants of a city by telephone directories and asked them to participate in a study (even if biases are likely because of selective willingness to participate) is the study of Rujescu and colleagues (2003).

A further problem correlating with some problems already mentioned, is the *quasi-experimental design*. Of course we have to measure individuals with the genotypes they were born with, so there are diverse kinds of moderating variables which could correlate with genetic features and variables we measure, but not in the way we suppose. For example, genes could influence emotions, which influence performance

or physiological variables (for example it is known that fear flattens the fMRI signal through maximal opening of arterioles during both experimental and basis condition). Or genotypes can seek specific learning environments (see also chapter 1) which can form their cognitive apparatus.

3.1.2 Methodological Issues of the Actual fMRI Study

fMRI (like other physiological measures, e.g. EEG) is a very powerful tool to investigate psychological functions like memory. It is very sensitive and yielded in our studies as in other studies reproducible activation patterns correlated with remembering (in the case of the prion study, recognition). Compared to a low-level baseline (comparing the first and last letter of a letter string), activations were much stronger in the left hemisphere than in the right hemisphere in practically all subjects. This baseline nicely subtracted out early visual activations and yielded activations where you would expect them (in the inferior frontal gyrus, BA 47 on both sides, in the dorsolateral frontal cortex BA 9, 8 and 6 left, the angular gyrus (BA 39) left; the middle temporal gyrus (BA 21) left, the basal ganglia on both sides and sometimes the posterior cingulate; see supplementary figure S2 of the paper). Choosing a higher-level baseline showed also the (“missing”) mediotemporal activations, rather parahippocampal than hippocampal, but sometimes also extending into the hippocampal formation (see Figure 24 as an example).

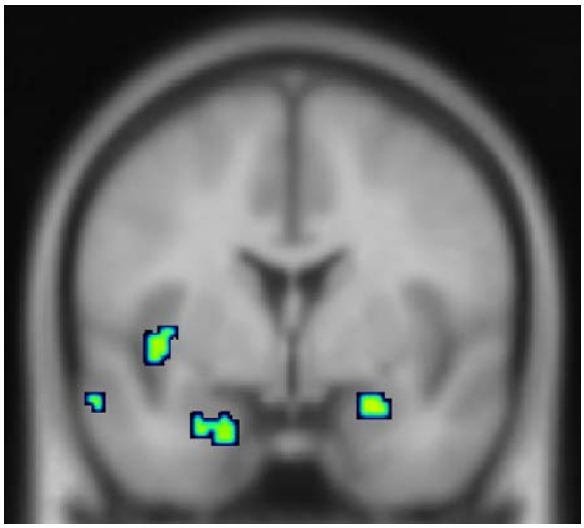

Figure 24: Activation in the whole sample (n=36) during recognizing the words learned 24 hours ago (only recollection), compared to the false positives as baseline; family-wise error corrected

The stronger activation of the parahippocampal gyrus than the hippocampus (Figure 24 is slightly misleading in that respect, which is the danger in single-slice figures; this is why I chose glass brains in the paper) is probably due to the single word learning task: Word *association* tasks (learning which words were on the same slide) challenge the hippocampus stronger. Often memory fMRI studies fail to show hippocampal activation, often due to inadequate baseline (see Stark and Squire, 2001): The problem is not that the hippocampus is inactive during remembering, but that he is active if subjects are bored and remember things to be entertained ('wandering mind').

The problem of using fMRI comparisons *between subjects* is that the signal is influenced by numerous factors other than the experimental manipulation (in our case, the genotype, which can be 'manipulated' only by selection), which strongly reduces the power as compared to comparisons *within subjects*. This is because many variables influence fMRI activation, which are controlled if conditions are compared within person, but which act as noise if conditions are compared between persons (for example brain anatomy or timings and form of the hemodynamic response function HRF). Good fMRI designs put all investigated conditions into the same time-series, which is of course not possible if comparing different genotypes.

An important issue in doing fMRI studies with genetics is also to use well-characterized tasks. In that respect, it is probably not a bad idea to take an

experiment that has been used several times already, because this facilitates the interpretation, especially if you do not see genotypic differences. The reason why we had to construct a new experiment was that the findings of Papassotiropoulos and colleagues (2005) had appeared in the word task and only in the 24 hours condition, so we had to construct an experiment involving a recall after a time-lag of 24 hours. To find optimal conditions and to test the experiment before applying it to the genotyped subjects, we tested already 28 subjects in pilot studies. Including a 24 hours recall strongly increased the need for experimenters. To compensate dropouts, we had to scan 50 subjects, which means about 60 hours of scanning, and 23.5-25.5 hours before scanning subjects had to learn the first word list, and half an hour before learning the second word list. This is why we needed up to 2 additional experimenters (blind for genotypes) per session to learn the word lists with the subjects. The work of these experimenters was quite critical, because one mistake would lead to one dropout. Another important reason for dropouts where subjects who repeated the words at home (and it was quite evident for them that they would have to remember the words from the first day on the second day, because all of them had already done an experiment from our collaboration partners involving a 24 hours recall). It was wise to schedule the neuropsychological session before the main experiment, because we could probably lower the number of drop-outs by excluding weakly motivated subjects from the study before using expensive scanner-time. The problem with dropouts was that our pool of genotype subjects was shrinking with every dropout, and we could not afford to do additional genotyping.

Another difficult issue to decide was the choice of a traditional *block-design* or an *event-related design*. One need was to maximize power, (which is especially difficult in a memory task where you can not make subjects learn 200 or more items) which would have spoken for a block design. However, assessing recognition with a block design has at least two disadvantages: One is that subjects may realise that there is a block-wise regularity in the sequence of the conditions (for example 4 learned items, 4 new items, 4 items baseline and so on), the other is that it is not possible to separate correctly recognized from falsely recognized items in the fMRI signal. An additional advantage of an event-related design is that we were able to differentiate recollective recognition (‘Remember’-answers, Tulving, 1985) from familiarity-based recognition (‘Know’-answers). Studies say that despite the signal-to-noise ratio is higher in a block design (Dale and Buckner, 1997) which practically means that you

need more items to get the same signal-to-noise ration in an event-related design), the event-related design extracts more information from the signal curve than the block-design (Mechelli et al., 2003).

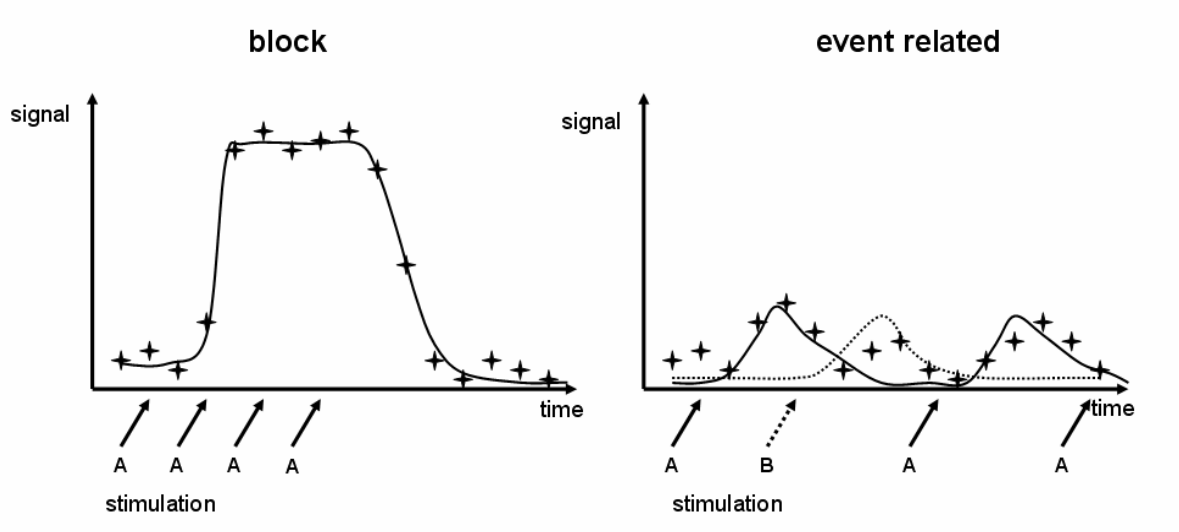

Figure 25: Comparison between block design and event-related design. The arrows designate stimulation (note that conditions are randomly or pseudorandomly mixed in event-related designs)

There are two differences of *fast event-related* (fast event related means that the next stimulus is given before the end of the HRF; otherwise you would have to wait at least 15 seconds to present the next stimulus) designs as compared to block-designs to be taken into consideration: (A) You have to make sure that you sample your full HRF, and (B) have enough time to do your statistics.
(A) Block designs assume that the signal is maximal for a large part of the duration of a block of about 20-24 seconds. If this assumption is correct, you do not have to worry much about the form of the HRF at its beginning or end, or about the timing of a measured slice with respect to the experimental variation (slice-timing correction is not necessary). In an event-related design, the form of the HRF has to be sampled, because otherwise you may miss maxima (which are rather narrow) and minima (which can be close to side-maxima). This means that you lose power, but even worse, you may measure artefacts of some regularity in the signal course (for example, if activation in condition B is always somewhat later than activation in

condition A; to account for this effect, we included time derivatives as effects of no interest, which shifts the beginning of all HRF's of a certain condition by an optimal amount; Josephs, 1997). Besides, slice-timing correction is indispensable, because otherwise you measure artefacts again, because one slice is measured at different times in the course of signal decay. The sampling of the whole HRF can be reassured with two preventive measures: First, use a length of an item that is slightly shorter or longer than the repetition time or an integer number x the *repetition time* (we used 8.82 seconds mean inter stimulus interval at a repetition time of three seconds, which means that during a time-series of 50 stimuli, the HRF was sampled three times; repetition time (TR) is the time of acquisition of one image of an entire brain) and second, use jittering. *Jittering* is a systematic delaying or speeding of an item (which has the additional advantage that subjects have to be more attentive not to miss a beginning), with the extreme case of omitting one or even two items (*null trials*). Jittering has been shown to make a design more efficient (Burock et al., 1998; Dale, 1999).
(B) Calculating the first-level fixed-effects model (for the within person contrasts, which are compared with other first-level contrasts in the group-analysis, which is normally done on the second level) is extremely time-consuming in an event-related design, because the vectors containing the time of stimulus-onsets are different from person to person. This means also that losing the behavioral data of a subject makes the imaging data worthless.

3.1.3 How to do it better? Important Points for Genetic Research

Knowledge-based search instead of screening:
Nowadays it is possible to compare 500000 or even more polymorphisms in a genome-wide search. This makes it possible to search for "the best memory gene" with relatively moderate costs (though, one chip costs some 1000$). In that way, you can compare for example the blood of 500 good learners with the blood of 500 bad learners. If you are lucky, you might find one polymorphism with a huge difference in frequency (see chapter 1.3.6). Otherwise the account does not help you because of the problem of multiple comparisons (see chapter 3.1.1). In most of the cases it is not possible to say that a difference exists, and at the same time it is not possible to say

that it does not exist. Therefore, it would be ideal to look at few well-characterized genes and polymorphisms in a hypothesis-based manner. Our studies assessed a very low level of organization (i.e. the polymorphisms) and a very high level of organization (i.e. the fMRI signal and the behaviour), but we do not know enough of the biochemical, developmental and neurophysiological processes inbetween. Therefore it would be ideal if an interdisciplinary institute, specialised for example in serotonin-correlated processes, would have methodological specialists to follow a path from the gene up to behaviour and again down to the gene. This institute would need knock-out mice, cell-cultures, ideally a developmental assay (for example chicken) and brain imaging (e.g. EEG and fMRI) in healthy volunteers and patients, maybe before and after clinical interventions. Then these specialists could build gene clusters and try to find out how they interact among genes and with environmental factors.

Work together:

A result in genetics is worth more if it has been found not only in independent populations, but in populations with different genetic backgrounds. Why do Japanese people resist longer to prion diseases? Why is the distribution of cancers different in different cultures? It would also be very helpful to use the same standards if possible (of course this is difficult with language test, for example) to get comparable results. Then one could do metastudies with larger numbers of subjects. Data pools should be accessible for any researcher: For example, one could do volumetric studies with enough structural MRI scans of persons of each genotype, age and sex (of course you can not match for all polymorphisms).

Involve more subjects:

In some countries there are population-based studies of certain topics, for example special diseases. The problem arises that healthy people are less willing to take part in a study or to have themselves genotyped. But it would be of great help to have data from healthy subjects as well: For example it would be very interesting to have data from persons who have a high genetic risk for a disease but do not get the disease. How are they different? How do risk behaviours interact with genetic risks? One important factor to motivate people to take part of our experiments is to do public work and inform all those who are interested about what we are doing and why.

3.2 Conclusions and Expectations in this Field of Research

While I tried to give an overview over some of the methodological problems in chapter 3.1, now I will try to speculate about the chances which this kind of research might hold.

In chapter 1.3, I briefly reviewed genes that are thought to be involved in memory and shortly summarized some results of these studies. As pointed out in chapter 3.1, I think that the aim of this kind of research should not be primarily to screen, confirm or disprove the involvement of genes in higher cognitive functions, but to build a chain from the gene through all organizational levels up to the behaviour and to show *how* the gene might influence behaviour. This is of course a great deal of work, but these kinds of results would give a yield, not only for basic science, but also for potential therapeutics: It is not wise to try to influence processes which we do not understand.

With respect to fMRI, this method should not be used as a hypothesis-generating, but as a hypothesis-testing tool. This is because fMRI is very *sensitive*, but sometimes not very *discriminative*. Below I will give an example of a case where it has been used very fruitfully in a genetic study (Heinz et al., 2004). But let me first summarize which of the studies cited in chapter 1.3 leave a better feeling of progress and which not.

For the 'clinical' genes PS1 and APOE, it is a valuable attempt to improve early diagnostics, which could turn out to be very important once a causal therapy against Alzheimer's has been developed. On the other hand, the gain of knowledge through our studies in these genes is relatively modest: The step from the gene to worse memory or increased fMRI activation is still too large to be understood. I would say something similar in the case of the prion protein gene Met129Val polymorphism: It might be really involved in memory, but what are the mechanisms? The numerous studies about the gene cited in chapter 1.3.7.5 give us a nice glimpse on what this protein could do, but it was probably too early to test the polymorphisms in humans, because no one knows what the polymorphism does on a cellular level. There is far more literature about the pathogenic mechanisms, but even they are hidden in the dark. To say it in different words, I'm sitting on quite promising results, but I am not able to give a hint of what they could mean, and probably no one is at this moment.

The situation is considerably better with the BDNF gene: There are not only concrete ideas about what this neurotrophin does, but it has also been characterized what the polymorphism does. This sets the stage for asking further questions (which should be assessed rather in animals than in humans) about how the different distribution of the protein influences brain structure and brain function. The 'best' gene mentioned in chapter 1.3 is probably the serotonin 2A receptor gene: I think that the involvement of this transmitter in memory has been shown convincingly, and the influence of the polymorphism has been shown on a cellular level. Memory seems to be quite sensitive to changes in serotonin transmission, not only between genotypes (de Quervain et al., 2003), but also upon slight changes in diet (Van der Veen et al., 2006). Probably it is a good idea to investigate genes involved in neurophysiological processes, glutamatergic or Gamma-Amino-Butyric Acid (GABA)-ergic transmission. Because of its probable involvement in overnight memory consolidation, acetylcholine would also be very interesting. An especially promising case are genes involved in monoamine (dopamine, norepinephrine, serotonin) transmission, because they act in larger parts of the brain more or less in parallel and are very well characterized by pharmacological studies because of their implication in psychiatric diseases. Above I promised to introduce a study which I find an instructive example of interdisciplinary genetic research:

In an earlier study, Lesch and colleagues (1996) showed that subjects with a shortened promoter of the serotonin-transporter (5-HTT), which leads to a diminished concentration of the transporter of about 50%, compared to the long variant of the transporter, score significantly higher on anxiety measures in personality inventories (the long/ long variant is found in about 31% of caucasians, the long/ short variant in 51%, and the short/ short variant in 17%). Interestingly, one short allele already increased the neuroticism score as high as two short alleles (besides, the short allele also reduced agreeableness of the subjects slightly, but significantly, with a similar to the "long/long > short/long = short/short" pattern observed in neuroticism). In a study with fear conditioning in humans, Garpenstrand and colleagues (2001) showed that carriers of the short allele learned an association between a neutral stimulus and an electric shock faster than homozygous long/long carriers (as measured by galvanic skin response). That this difference had to do with serotonin metabolism was further implicated by the fact that the effect was more pronounced in subjects who expressed little monoaminoxidase, an enzyme that splits serotonin and therefore

removes it from the synaptic cleft (like the serotonin transporter 5-HTT, which allows the presynaptic neuron to efficiently reuptake serotonin). Besides, a longer variant of the third exon of the dopamine D4 receptor slowed extinction of the fear response. In a longitudinal study, Caspi and colleagues (2003) could show that though the polymorphism is not proven to be associated with depression, it is associated with the response to stressful environments: Despite statistically equal number of stressful life events between genotypic groups, life events predicted (a) symptoms of depression (whether self-reported or by a nearby person), (b) a depression diagnosis and (c) suicide thoughts or attempt only in carriers of at least one short allele. Early depression (earlier than at an age of 20) was excluded from the analysis.

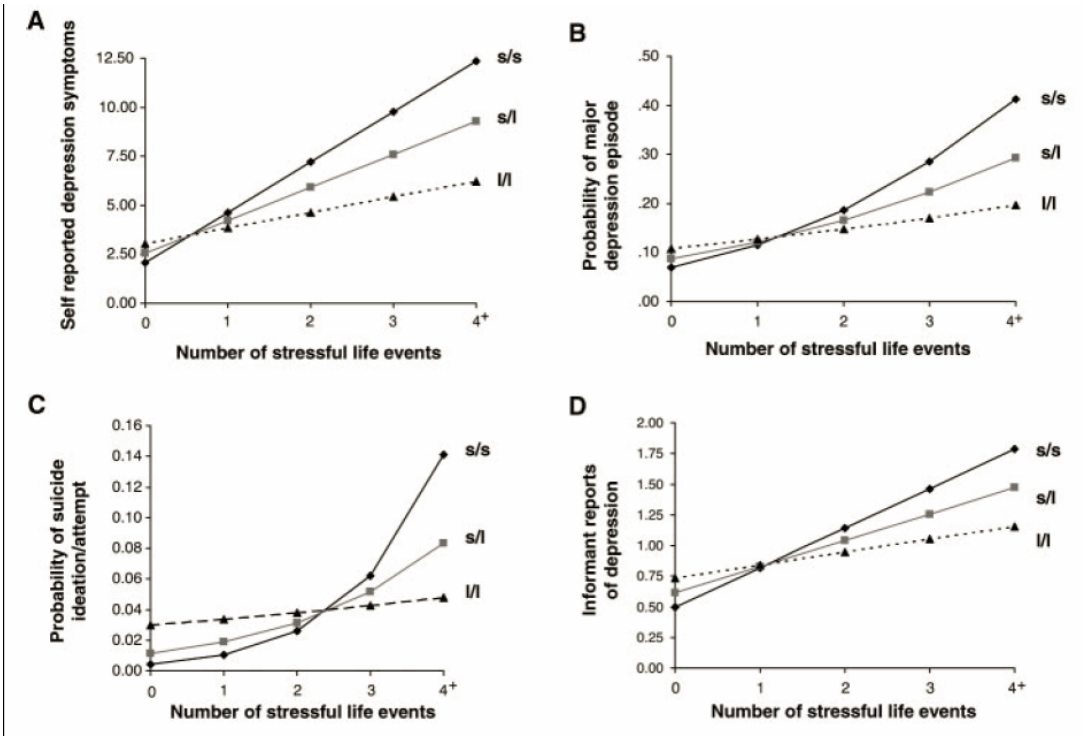

Figure 26: Relation between 5-HTT genotype, life events and depressive symptoms (from Caspi et al., 2003).

A further study showed a stronger amygdala activation upon fearful stimuli (Ekman faces) in carriers of at least one short allele of the 5-HTT transporter promoter polymorphism (Hariri et al., 2002; studies with depressed patients have shown the same amygdala overactivation, which improved after antidepressive medication; see Drevets et al., 2003). In a further fMRI study, Heinz and colleagues could show that aversive stimuli activated both amygdalae, stronger in carriers of the short allele than

in long/long-carriers (more pronouncedly in short/ short carriers), which was not the case for pleasant stimuli. More importantly, the coupling (coactivation) between the amygdala and the ventromedial prefrontal cortex on each side (BA 11 left, BA 10 right) was much stronger for carriers of at least one short-allele than for homozygous long/ long carriers (see Figure 27).

The authors discuss this finding in terms of a higher capacity for short allele carriers to regulate their emotional responses with their prefrontal cortex, which does not necessarily indicate greater vulnerability, but may lead to a problem in stressful situations, where this coupling might be too rigid and stabilize mood on a low level. While the interpretation seems to me a bit superficial (why exactly is coupling sometimes good, sometimes bad?), the finding is intriguing, since it could let the above findings converge and tell us why there is a faster fear conditioning in carriers of the short allele.

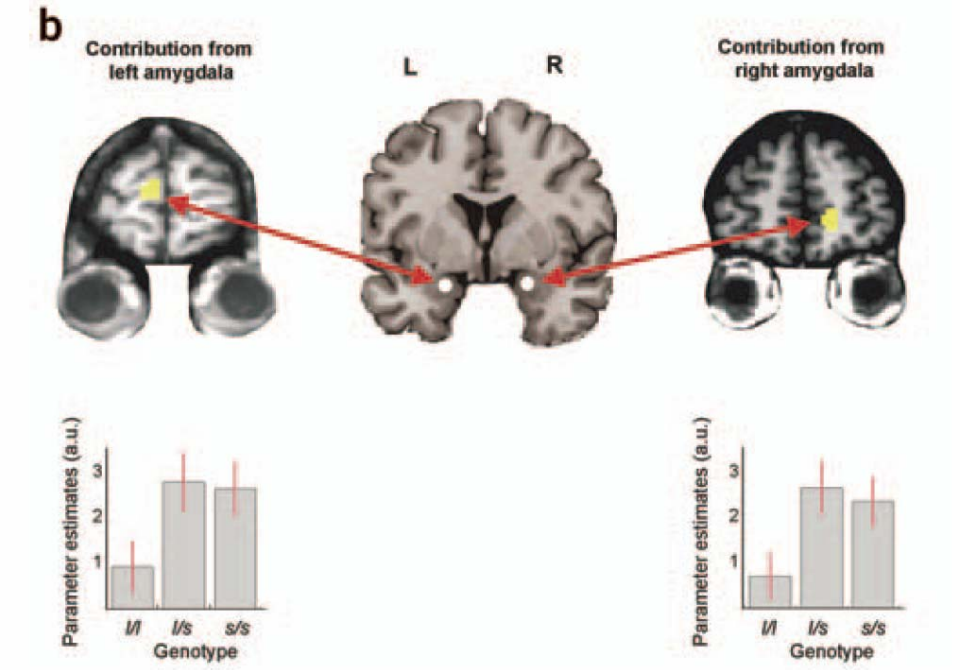

Figure 27: Stronger amydala-prefrontal coupling in carryers of a short allele of the serotonin transporter gene promoter (from Heinz et al., 2004)

What does this example show? First, using naturally occurring polymorphisms can allow us to investigate disorders directly in humans, for which we otherwise would have to use animal models, from which we never know if they are valid models for the disease (depression and schizophrenia are especially difficult to model). Second,

fMRI can help us a lot if we have a priori hypotheses of involved processes (in this case, the amygdala activation upon aversive stimuli): Now we have a model at hand which we can test in depressed subjects: Is the amygdala-prefrontal coupling really greater in people at high risk for depression than in people with lower risk for depression? Is it only part of the diathesis, or is it also a consequence of the stress? Is this coupling a trait, and if yes, does the trait predict depression after stressful events? Of course it would be important to characterize the psychological correlate of the coupling. Maybe it could elucidate the old controversy about what is first in depression- the emotion or the thought (probably this is the wrong question to ask).

To say it in other words, ideally we should not set up to “do a genetic study”, but use genetic tools to elucidate concrete, well-characterized research questions, together with other tools.

Another very important field for genetic tools (beside neurophysiology) is probably brain development (see e.g. Balaban, 2006). For example, from six genes implicated in developmental dyslexia, four are involved in the radial migration of neurons (reviewed by Ramus, 2006). What needs the brain to configure itself in a way that enables learning? Are Brodmann’s areae prespecified? What cues are needed for a circuit to develop? Of course this is rather research for biologists than for neuropsychologists. But there is enough to do for us between brain and cognition: Which concepts are useful in both? Which psychological function does a brain area execute (see chapter 1.2.3.1)? And if a biologist has made the observation that protein x influences strongly cell-type Y, which is found mainly in brain area A, and a specialist in genetics tells us that the gene X in humans has a common polymorphism that changes the activity of protein x, then we are ready to set up a design to test if the polymorphism in X really influences function a of brain area A, and our results potentially answer two kinds of questions, namely (1) if we discover a similar effect in humans than was found in animals and (2) we might be able to further characterize the functions of brain structure A. This is what I mean with concrete research questions and with interdisciplinary work.

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Curriculum Vitae

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Education

1981-1987	Primary school
1987-1993	High school
1993- 1995	Physics (undergraduate) University of Zurich, Switzerland,
1996- 2003	University of Zurich, Switzerland Main subject: Experimental Psychology 1. subsidiary subject: Psychopathology 2. subsidiary subject: Neurophysiology
2003-2006	PhD in Neuroscience, Center for Neuroscience, University of Zurich

Appointments

Feb./ Mar. 2000 (7 weeks)	Nurse training in the Psychiatric Clinic of Herisau, Switzerland
Sept./ Oct. 2000 (7 weeks)	Medical training in the Psychiatric Clinic of Schaffhausen, Switzerland
Feb.-Apr. 2002 (13 weeks)	Internship in Clinical Neuropsychology at the Swiss Epilepsy Center, Zurich, Switzerland

Jul. 01 - Jan. 02 and May/ June 02 (totally 8 months)	Civil service in the “Blindenwohnheim Mühlehalde” in Zurich, Switzerland: 7 months care and 1 month nursing
Dec. 2003 -	PhD in Neuropsychology and Imaging at the Division for Psychiatry Research, University of Zurich, Switzerland. Supervisor: Katharina Henke

Clinical Neuropsychology Expertise

2003-	Clinical neuropsychological examinations at the Ambulatory Care Unit of the Division for Psychiatry Research, University of Zurich. Clientele: Patients with neurodegenerative disease. Supervision: Katharina Henke, Henrietta Mustovic
Feb./ Mar. 2002	Thirteen weeks of diagnostic training during internship at the Swiss Epilepsy Center in Zurich. Clientele: Children with learning problems and epileptic patients of any age. Supervision: Hennric Jokeit

Teaching Experience

1993-	Astronomical Presentations, Sternenberg Observatory
1997-2001	Statistics Tutor, University of Zurich, Institute of Psychology
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